THE UNIVERSITY OF ALBERTA

SUSCEPTIBILITY OF SPECIES OF MEDICAGO TO BRUCHOPHAGUS RODDI GUSSAKOVSKII

by

JAMES HADLAND THOMAS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Susceptibility of Species of Medicago to Bruchophagus roddi Gussakovskii," submitted by James Hadland Thomas, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Differential resistance of 60 Medicago species to Bruchophagus roddi Guss. was demonstrated. Essentially all 25 perennial species tested and 12 of 35 annual species tested were infested by this pest, but at significantly different rates.

A study of pod characteristics was made in relation to infestation by crossing a resistant species with several susceptible species and exposing the F_2 plants to Bruchophagus roddi. The results showed that the insects infested tight, many-curled pods less than pods that are open, i.e., whorls not close together, and having few curls.

It was demonstrated that the same biotype of Bruchophagus roddi infested both Medicago polymorpha L. (bur clover) and Medicago sativa L. Insects from either of these Medicago species were shown to infest the other at equal rates.

Prolonged storage of overwintering larvae of Bruchophagus roddi effected emergence. Mortality increased with time in storage but at different rates for males and females. After 36 months in storage the ratio of emerging males to females was 1:7.

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James H. Thomas

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The alfalfa seed chalcid, Bruchophagus roddi Gussakovskii, a small, jet-black wasp, is a serious pest in alfalfa seed production, and one for which no satisfactory control has been developed.

The damage done by this insect has been known since the early 1900's, but the extent of the damage and loss in product and revenue has increased to alarming proportions in the past 15 years with the specialization of agriculture. Losses due to infestation by the chalcid have been estimated in recent years by some growers at 60 percent (Strong, 1960) of an entire seed crop.

The pest is found wherever alfalfa is grown for seed, but is especially prevalent in the Southwestern United States where arid conditions and long warm seasons are conducive to its development.

Cultural, chemical, and biological controls have been investigated, and some success in each of these areas has been reported. However, the life cycle of the pest, and the close relationship in field activity to the pollinators that are required for seed set, has made the above methods largely ineffective.

Parasites of the chalcid have been studied, but the parasites must be raised in vivo, and it is not known whether there are any specific predators of the adult chalcid.

The method of control that seems to hold the greatest possibilities at present is selection for resistance, and some progress

has been made in this field. Investigators in the United States have screened thousands of plants of commercially grown alfalfa, plant introductions, and a few Medicago species (Nielsen, 1966). Resistance has been found. However, none of the plants selected for resistance are immune, i.e., completely free from infestation under all circumstances, and a "resistant variety" when introduced would only be "less infested" than others. Insect adaptation could conceivably nullify any gain in a short time.

With these facts in mind, it was decided to investigate the species in the genus Medicago to see: 1) if some of the species were immune, 2) if those species which were infested showed differential resistance, and 3) if immune or resistant species could be developed into a commercially useable forage.

LITERATURE REVIEW

Classification of the Alfalfa Seed Chalcid

The alfalfa seed chalcid was probably first described in England in 1834 by Walker, who named it Systole platyptera, and in Sweden in 1836 by Boheman, who named it Eurytoma gibba (Peck, 1963). The first description of the chalcid in North America was by Howard (1880), who considered it a parasite of the seed midge, and named it Eurytoma funebris. Ashmead (1894) transferred the chalcid to the genus Bruchophagus, believing it parasitized seed weevils (Bruchidae). In 1896, Hopkins (1897), after careful observations, found the chalcid was a pest of clover seed. The pest was known for some time as Bruchophagus funebris and then Bruchophagus gibbus (Boheman).

Since more intensive investigation of the pest was started in the early 1900's, the name of the insect has undergone numerous changes, and reports have indicated probable hosts ranging from other insects (Viereck, 1910) to the seeds of plants in six genera and at least fourteen species, mostly legumes (Sorenson and Knowlton, 1951; Neunzig and Gyrisco, 1958; Strong, 1962a). For many years the chalcids emerging from trefoils, Red clover, and alfalfa were considered a single species. From 1880 to 1893, these chalcids were generally reported as Eurytoma funebris; from 1894 to 1931 they were classified as Bruchophagus funebris; and from 1932 to 1955, Bruchophagus gibbus was the name given to these pests in

legumes (Peck, 1963). Frequently the older names were reverted to, or host names were included, i.e., Bruchophagus gibbus medicaginis (Guppy, 1958), indicating those chalcids infesting Medicago species.

Recent morphological and biological studies have shown that what has been called the clover seed chalcid Bruchophagus gibbus is actually three distinct species, Bruchophagus gibbus, Bruchophagus roddi, and Bruchophagus kolobovae (Kolobova, 1960; Nikol'skaya, 1932, 1952; Fedoseeva, 1958; Strong, 1962a; Batiste, 1964). Strong (1962a) reported that separation of these three species is possible on the basis of the female genitalia. Batiste (1964) devised a quick method of differentiating between Bruchophagus kolobovae and Bruchophagus roddi, using the height:length ratio of thorax measurements.

Investigators started naming chalcids according to the host infested about 1955. Two of the names used were introduced much earlier: Bruchophagus gibbus in 1932 and Bruchophagus roddi in 1933. Bruchophagus kolobovae was described in 1956 as that species infesting Lotus species.

The present classification is as follows: Order Hymenoptera, superfamily Chalcidoidea, family Eurytomidae, genus Bruchophagus, under which there are three specific names depending on the host the species infests. The species infesting trefoil (Lotus species) has been designated Bruchophagus kolobovae (Fed.). The species infesting clovers (Trifolium species) has been designated Bruchophagus gibbus (Boh.). The species infesting alfalfa has been designated as Bruchophagus roddi (Guss.).

This thesis is concerned exclusively with the "alfalfa" seed chalcid, and any reference to "chalcid" means Bruchophagus roddi (Guss.) unless otherwise designated.

Control

Cultural methods of controlling the chalcid are based on an understanding of the life cycle. Sorenson (1930) and Urbahns (1914 and 1920), describe field practices that they felt would reduce the number of chalcids throughout the season. They include the following:

1. Keep volunteer plants along fences, ditch banks, and around buildings from setting seed, and eradicate bur clover where possible. Volunteer plants and bur clover (Medicago polymorpha and Medicago arabica) serve as host plants when field plants are still maturing, and chalcids emerging from these "fence-line" plants are the initial source of infestation in the growing season.

2. Chaff stacks and screenings from the previous season's crop should be burned or utilized in stock feeding to prevent chalcids overwintering in the seed (in diapause) from emerging to reinfest current crops.

3. Seed producers in a given area should co-operate and all grow the same crop of alfalfa for seed. This prevents chalcids emerging from one field from infesting nearby fields that will mature later.

4. After harvest, fields should be cultivated and irrigated to destroy larvae in diapause.

Bacon et al. (1963) conducted experiments to evaluate the effectiveness of these suggestions. These investigators tested burning, irrigation, and cultivation as control methods. Burning had little or no effect, but irrigation, and especially cultivation, produced significant reduction in emergence. Chalcids in seeds that were covered by 0.5 inches of soil failed to emerge.

There are insecticides available to the public as well as numerous experimental chemicals that are lethal to the adult chalcid (Shabbir, 1961), but effectiveness of these when applied during the period of bloom and seed set has not been proven. Crothers (1962) and Bacon et al. (1963) used several systemic insecticides, but these were only effective on adults and not on developing larvae. Eggs laid prior to application developed normally.

The use of chemicals in field practice has not proven feasible, however, because chalcids are present in the field and on the plants at the same time that pollinators are, and use of insecticides eliminates a large portion of the essential pollinators. Anderson et al. (1961) have shown that insecticides that are highly toxic to chalcids are also highly toxic to honey bees. Insecticide effectiveness is further reduced because chalcids emerge intermittently all season. Bacon et al. (1963) have shown that insecticides are effective only as a soil treatment and then only when the soil is wet at the time of application.

Several investigators have reported on chalcid parasites (Nikol'skaya, 1932; Neunzig and Gyrisco, 1958; Butler and Hansen, 1959; and Tilley, 1960). Ten or more species are discussed and reported as parasitizing the seed chalcid complex, with different parasites more prevalent in some areas, and some degree of host specificity shown. Most of these parasites attack the larval stage. A "biological" control program involving increase of chalcid parasites would not prevent infestation, but would reduce future numbers of chalcids. This type of control is not feasible at present because chalcids for hosts cannot be grown in vitro.

Life Cycle

Adults. Adult chalcids emerge from over-wintering seed 10 to 15 days after the onset of consistently warm weather in the spring. Since the actual time of the year varies with the latitude, no general date can be given. Male adults emerge from 2 to 4 days before females, and are ready to mate as soon as the females emerge. Mating usually takes place within a few hours of female emergence, and oviposition begins, if suitable pods are available, within 24 to 36 hours. Strong (1962) has shown that the oviposition period may extend to two weeks, and that the egg laying capacity averages between 35 to 45 eggs, with exceptional females laying over 80 eggs. Sorenson (1934) states that females can fly "long" distances to find pods suitable for oviposition, and can "wait" several weeks if none are available.

Oviposition. Batiste (1964) has described the ovipositional activity of Bruchophagus kolobovae, and Bruchophagus roddi were observed to exhibit essentially the same habits. The female locates suitable seeds within the pod by "tapping" with her antennae. When a seed is located the abdomen is lowered, the ovipositor inserted, and an egg is deposited in the interior of the seed. Seed thus penetrated can be identified within a few hours as there is brown scar tissue formed at the probe site. In many seeds several probe scars were observed, but only one larva was present. This agrees with Sorenson's (1934) estimate that less than 2 percent of the infested seeds have more than one larva. Only 3 seeds out of 400 dissected in this study were doubly infested.

Batiste (1964) indicates that Bruchophagus kolobovae females show territorial behavior. This was also observed in Bruchophagus roddi. One female when watched over a two hour period, repeatedly drove off approaching females from "her" pods.

Egg. The egg is milky-white when laid, but gradually clears to nearly translucent by hatching time. Incubation normally lasts from 1 to 3 days (Strong, 1962).

Larva. A small, white, legless grub hatches and the larvae begin feeding on the seed embryo and cotyledons. Feeding continues through 4 instars and takes 12 to 13 days.

Diapause. A chalcid larva does not defecate during its development (Strong, 1962). This is common in hymenoptera and is

probably a mechanism to prevent adverse effects of fecal material discharged in hosts resulting in soiling the food supply. After the larvae have reached maturity, i.e., at the end of the 4th instar, they defecate and then enter the pre-pupa stage. If chalcids enter diapause they do not defecate; diapause larvae can be identified by the presence of feces in the gut.

Pupa. The pupa stage takes from 12 to 13 days during which the pupae change from white to black. The adults emerge within a few hours of reaching maturity. A hole is eaten in the seed coat and pod if the seed is unshelled.

Total time from egg oviposition to adult emergence under normal conditions is 27 ± 4 days (Strong, 1962). The length of time from defecation after diapause to emergence, as determined by emergence records, varies considerably. It is affected by prolonged diapause resulting from storage of the diapause larvae at cool temperatures (see emergence studies section for details).

Resistance

"Plants which are inherently less damaged or less infested than others under comparable environmental conditions in the field are called resistant" (Painter, 1958).

Painter lists three basic components, and suggests that resistance may be the result of these components acting singly or in combination. Firstly, plants may be "non-preferred" due to a

lack of qualities favorable for oviposition, shelter or food, or due to the presence of some repellant. Secondly, "antibiosis" may be in evidence, wherein the resistant plant adversely affects the biology of the insect, or its offspring, resulting in reduced populations. Thirdly, resistant plants may be "tolerant" in that they can undergo attacks by the insects and still develop or reproduce or both where a susceptible plant would die or fail to reproduce. Painter considered this third characteristics a component of resistance even though the insect may not be repelled in any way by the plant.

Plant resistance to the alfalfa seed chalcid has been reported by several investigators (Bunker, 1959; Minion, 1961; Rowley, 1962; Thomas, 1963). Selections have been made in an attempt to produce a synthetic variety of alfalfa resistant to the chalcid, for use in areas where chalcid damage has been excessive (Nielsen, 1966).

In an attempt to determine some reasons for the differences in infestation among several susceptible or resistant varieties of commercial alfalfa, the author (1963) compared pod wall thickness and the number and "tightness" of curls per pod with infestation rates. It was found that thick pods correlative positively with high infestation and number of curls per pod and the tightness of the curls correlate negatively with high infestation.

Work has been initiated to determine if there is a biochemical basis to susceptibility or resistance (Haws, 1966).

To date the majority of resistance studies have been made on commercially grown tetraploid varieties of Medicago sativa and Medicago falcata. Several other species of Medicago have been reported as being infested by Bruchophagus roddi (Strong, 1962; Schaad et al., 1952). These include Medicago polymorpha, Medicago arabica, and Medicago minima, all of which are annuals, and the perennials Medicago agropyretorum, Medicago prostrata, Medicago lavrenkoi, Medicago parviflora, Medicago gaetula, Medicago glutinosa, Medicago polychroa, and Medicago mesopotamica. The literature leaves us uncertain as to which species of Medicago are actually hosts to the chalcid. The research in this thesis is an attempt to clarify this problem, and to determine whether some of the host species are more resistant than others.

MATERIALS AND METHODS

Greenhouse and Growth Chambers

The majority of the experiments were carried out in the greenhouse and in growth chambers. In the greenhouse during winter months the light period was maintained with supplemental lights at 16 hours to induce blooming of the plants. Relative humidity was generally near 70 percent, and the temperature was 68° F. if there were no insects being used, but 75° to 80° F. if insects were in the compartments.

The growth chambers were the walk-in type, 9 X 30 feet. They were equipped with General Electric "power-groove" fluorescent tubes emitting light from 3300 to 7200 Angstroms, and giving 2200 candlepower at bench height. The tubes served as a heat source and the chambers were equipped with cooling, circulation, and exhaust systems, to maintain optimum growing conditions. Plants in the chambers bloomed readily and maintained a healthy appearance if proper fertilization and insect control were practiced.

Growth chamber conditions varied only slightly from the greenhouse conditions. When insects were being used in the chamber the temperature was kept near 80° F. to promote insect activity.

Insect Cages

Several types of insect cages were tried, depending on the plants being tested, whether the plants were in pots or flats, and

how much freedom was desired for the insects.

Individual pot cages. When several plants were grown in a single pot and numerous insects were placed on the plants for infestation, a cage was made of organdy supported by a frame of number 9 construction wire, and tied around the base of the pot. The organdy was approximately 80 mesh; it confined the insects, yet allowed air circulation. Watering was done by momentarily lifting one edge of the cloth or by using watering trays under the pots.

Free choice cages. Cages 3 X 3X 3 feet were constructed from plastering lath and 1 X 1¼ inch boards. The frames were open on all 6 sides and were covered on the left, right, and back sides with clear 2 mil polyethylene plastic stapled to the frame. The top and bottom were covered by 80 mesh organdy to permit air circulation. On the front a sheet of flexible 6 mil plastic was mounted, to permit access to the cage for placing pots or flats in it, or for watering. Masking tape was used to make the cage "insect tight." These cages were large enough to hold sixteen 5-inch pots or two 4 X 14 X 24 inch flats. These cages were used when the insects were given free choice of numerous plants.

Individual raceme cages. *Drosophila* breeding bottles were used to confine insects to a single raceme. The bottles measured 1 X 3¾ inches and were clear glass or plastic and open at one end. Absorbent cotton was fitted around the stem or pedicle and the bottle containing the chalcids was then pushed over the pods and down to the cotton, effectively stopping the bottle.

When two racemes of different plants had to be put in the same vial for a controlled test, a glass tube 1 X 4 inches was used. One raceme was inserted into each end and cotton was used to stop the ends.

Control Plants

Preliminary experiments showed that a male sterile plant of Medicago sativa known as 20 DRC (Childers) was readily infested by Bruchophagus roddi. It has large, only slightly curled pods, many seeds per pod, and 12 to 18 flowers per raceme. Being easily propagated from cuttings it was used as a control plant in all the following experiments.

In the large free choice cages, a pot of 20 DRC was included in each cage of test plants. Pollen from regular tetraploid alfalfa was used to fertilize 20 DRC. When an individual pot cage was used a control plant was caged with insects at the same time.

In some cases control plants were not used. In tests between two or more plants that ordinarily have low rates of infestation, the control was omitted so that all the insects would not be attracted to the control plant.

Pollination

Wild bees and honeybees did not have ready access to the greenhouse and pollinating insects could not be used in the growth

chambers, so all perennial plants used in the experiments conducted in the greenhouse or growth chamber were hand pollinated. A razor blade was used to modify the end of a flat toothpick which was used to "trip" the plants. A different toothpick was used for crossing each strain or species of plant, and care was taken not to use "strain-foreign" pollen so that seed purity would be maintained.

Generally hand inter-pollination was satisfactory. Approximately 60 percent of the flowers tripped produced pods. This varied however, as some plants produced nearly 100 percent pods from cross-pollinated flowers, and others produced no pods when pollinated with pollen from their own strain. In the latter cases pollen from a different strain of the same species was used, and the seed produced was not kept for further use.

All the annuals used were self pollinating.

Labelling

Small white marking tags fitted with cotton string were used almost exclusively. Large tags of different colors were used in the crosses. Tags were not used in the vials, but a felt pen was used to identify the plant by writing on the vial when it was removed.

Painted wooden or plastic pot-stakes were used to identify plants in pots or flats.

Germination

Most of the seeds used in this study were hard and would not imbibe water. To induce germination before planting, the seeds were scratched on a piece of fine sand paper or emery cloth. They were then placed inside a petri dish on a filter paper, moistened with distilled water. To prevent growth of molds commonly associated with legume seeds, a thin layer of sand treated with Arasan was put in the bottom of the petri dish before the filter paper was put in. The dishes were placed in a small refrigerator at a temperature of 50° to 55° F., and the seeds allowed to germinate. Most seeds germinated within a week of being scratched, but some germinated only after several weeks.

When it was evident that seeds were germinating, they were planted in pots or flats.

Soils and Fertilizers

Black loam supplied by the University Greenhouse was used in all experiments. A mixture of 3 parts soil, 1 part sand, and 1 part peat moss was used throughout, and superphosphate fertilizer was added initially to the soil mix in sufficient quantities, as determined by soil tests, to bring fertility up to the optimum level.

Subsequent fertilizer additions were made in the form of a soluble fertilizer additive, generally a commercial preparation of either Plant Prod (Analysis 15:30:15) or Soluble Shur-Gain (Analysis 10:52:17).

Insecticides

Greenhouse and growth chamber insect pests were controlled by periodic use of Kelthane, Diazinon, and an experimental chemical called Zectran. Malathion dust was used occasionally to control red spider mites.

Care was taken to avoid using insecticides with long residual action if the plants were near the stage when they would be exposed to chalcids.

Insect Material

Alfalfa seed infested with Bruchophagus roddi was obtained from several investigators in the United States. Two 100-pound shipments came from Davis, California, one harvested in 1965 and one in 1963. Three 50-pound lots came from Prosser, Washington, and three 20-pound lots from Tucson, Arizona. In addition to this, three shipments of fresh pods were obtained; one of bur clover (Medicago polymorpha), and one of alfalfa from Tucson, Arizona, and one of bur clover from College Station, Texas.

The infested seed was obtained in all cases from commercial alfalfa seed cleaning plants near the locations mentioned above. The fresh bur clover pods were collected in uncultivated areas or golf courses, and the fresh alfalfa pods were collected from a commercial field.

Handling of Insects

As soon as the infested alfalfa seed was received, it was stored in a cold room at 43° F., and 66 percent relative humidity. Approximately three weeks before insects were required, seed was removed from the cold rooms, placed in plastic watering saucers (8.5 inches in diameter, 1.5 inches deep), covered with some of the organdy material mentioned previously, and kept in an incubator at approximately 75° F. When insects were needed the trays were removed from the incubator, uncovered in subdued light to prevent their escape, and a piece of glass was placed over the tray permitting counting.

The infested bur clover seed, still in the pods, was kept in emergence cages similar to those used by Strong (1962) and Batiste (1964), made from icecream cartons and plastic or glass vials. Chalcids were used as they were collected in the vials.

Insect Parasites

Several species of chalcid parasites emerged from the infested seed material. The most common was Liodontomerus perplexus as identified by Butler and Hansen's (1959) description. The parasites did not begin to emerge until most of the Bruchophagus roddi had emerged, but care was taken near the end of the emergence period to cull out the parasites.

Chromosome Counts

Since some of the accessions of some Medicago species were of unknown ploidy level, the chromosome counts of these were undertaken. The method used was one suggested by Lesins and Lesins (1963).

Plant Materials

All the plant material used in the experiments was provided by Dr. Karl Lesins from the collection he maintains in the Department of Genetics, University of Alberta, Edmonton. Tables 1 and 2 show the species of perennials and annuals respectively, that were available for the experiments.

Statistical Methods

All infestation percentages were converted using an arcsin table (Snedecor, 1956) and analysis of variance was performed on the converted values. Duncan's (1955) Multiple Range Test was used to show significant differences where numerous means could be compared, whether the F test proved significant or not.

Significant differences shown by the multiple ranges are at the 1 percent level only where the F test proved significant at that level; all others are at the 5 percent level of probability.

Table 1. Perennial species of Medicago available for the tests. Species arranged alphabetically.

Species	No. of strains	2n No. of Chromosomes
1. agropyretorum Vass.	2	32
2. arborea L.	3	32
3. cancellata M.B.	1	48
4. carstiensis Wulf.	2	16*
5. coerulea Less.	10	16, 32
6. daghestanica Ruprecht	1	16
7. dzhawakhetica E. Bordz	2	16, 32
8. falcata L.	29	16
9. glandulosa David	5	16
10. glomerata Balb.	1	16
11. glutinosa M.B.	1	32
12. hemicoerulea Sinsk.	2	16
13. hemicycla Grossh.	6	16
14. karatschaica Latsch.	1	32
15. lavrenkoi Vass.	1	16
16. leocarpa Benth.	1	16
17. marina L.	1	16*
18. papillosa Boiss	2	16
19. pironae DeVisiani	1	16
20. prostrata Jacq.	2	16*
21. rhodopaea Velin.	1	16
22. romenica Prod.	1	16
23. sativa L.	11	16, 32*, 48

Table 1. continued .....

Species	No. of strains	2n No. of chromosomes
24. *saxatilis* M.B.	1	48
25. *suffruticosa* Ramond	2	16*
26. *tenderiensis* Opperman	1	16
27. *vardanis* Vass.	1	32
28. *sativa* (2n=48) X *cancellata*	1	48

* indicates accessions in which chromosome number was determined during the study.

Table 2. Annual Medicago species tested showing 2n chromosome number for each. Species arranged alphabetically.

Species	2n Chromosome No.	Specie	2n Chromosome No.
1. aculeata Willd.	16	19. orbicularis (L.) Bart.	16
2. arabica (L.) Huds.	16*	20. polymorpha L.	14
3. aschersoniana Urb.	16	21. praecox D.C.	16*
4. blancheana Boiss.	16*	22. rigidula (L.) All.	14
5. ciliaris Krock.	16	23. rotata Boiss.	16*
6. constricta Dur.	14	24. rugosa Desr.	32*
7. coronata (L.) Bart.	16	25. sauvagei R. Negre	16
8. disciformis D.C.	16	26. scutellata (I.) Mill.	32
9. granadensis Willd.	16*	27. secundiflora Dur.	16
10. intertexta (L.) Mill.	16	28. sessilis Peyron	16*
11. laciniata (L.) Mill.	16*	29. shepardi Post	16
12. lanigera Winkl. et B. Fedtsh.	16	30. soleirolii Duby	16
13. littoralis Rhode	16	31. striata Bast.	16
14. lupulina L.	16	32. tenoriana Ser.	16*
15. minima (L.) Bart.	16	33. tornata (L.) Mill.	16*
16. murex Willd.	16	34. truncatula Gaertn.	16
17. muricoleptis Tineo	16*	35. turbinata (L.) All.	16
18. noeana Boiss.	16		

* Indicates accessions in which chromosome number was determined during the study.

RESULTS

Infestation of Perennial Medicago Species

In order to determine which of the perennial species of Medicago actually serve as hosts for the alfalfa seed chalcid, all the available species and strains were included in an infestation experiment.

Seeds from each of the 93 entries shown in Table 1 were germinated and planted in 5 inch pots. At least 4 pots, with 4 plants per pot were planted from each entry. The plants were grown to maturity in the growth chamber, and when they had sufficient blossoms to yield a reliable test they were intercrossed and each raceme was tagged. When the pods had begun to form, usually after the fourth or fifth day, the number of pods was counted and recorded on the tag, and the pot was placed in one of the free choice cages described earlier. A 20 DRC control plant was pollinated with tetraploid alfalfa pollen, tagged, and the pods counted and included in the cage. One cage conveniently held 16 pots (15 test and 1 control) so the plants were tested in groups of 15 pots made up of approximately 60 plants.

Chalcids were introduced into the cages at the rate of one chalcid per pod. The male to female ratio (see emergence data presented later) of 1:1 made two pods available for each female. Taking into consideration the "territorial" behavior of the females it was estimated that this ratio of pods to females

would give high enough infestation rates to determine not only which entries were actually hosts, but also if there were significant differences among strains within a species or among plants within a strain.

The plants were left in the cage for 14 days, then removed and allowed to mature for another 14 days. The pods were then harvested on a single plant basis, opened, and the percent infested seed was determined.

Two of the species did not blossom in the growth chamber, i.e., Medicago carstiensis and Medicago arborea. Four of the species produced insufficient blossom to allow testing, i.e., Medicago dzhawakhetica, Medicago papillosa, Medicago pironae, and Medicago rhodopaea.

All of the above species blossomed well enough in the field to allow testing with individual raceme cages, except Medicago pironae and Medicago arborea. Medicago arborea could not be induced to blossom under any circumstances in the experiments here and Medicago pironae blossomed in the growth chamber and in the field, but in both cases flowers were insufficient for determining the infestibility of this species.

Preliminary analysis of the infestation rates showed little variation among plants within a strain, so the infestation rates of all the plants in a pot were averaged, and since the four pots of each strain were tested in different cages, a pot was considered a replication.

Table 3 shows the percentage of seeds infested in the 89 entries included in the experiment. Twelve entries were not infested in these tests. Diploid Medicago coerulea appeared to be immune to the chalcid, but later tests proved it infestible. The same is true for Medicago sativa (509) which was not infested in these free choice cage tests.

Medicago falcata strains all had relatively high infestation. Over half of the 30 highest infested entries were Medicago falcata and all Medicago falcata entries were included in the first 55 of the 89 entries.

Medicago lavrenkoi, Medicago glutinosa, and Medicago prostrata were all generally highly infested. Medicago daghestanica, Medicago cancellata, Medicago saxatilis and Medicago carstiensis were infested at less than 5 percent of the seeds.

Four species of the perennials had a sufficient number of strains in the original tests to test for differences among strains. They were: Medicago sativa, 10 strains; Medicago falcata, 29 strains; Medicago glandulosa, 5 strains; and Medicago hemicycla, 6 strains. Medicago coerulea had 10 strains but was not infested in the original test, and is treated in a later section.

Analysis of variance was calculated on the data from each species: Medicago glandulosa showed differences among strains at the 1 percent level of probability, Medicago sativa and Medicago hemicycla each showed significant differences among strains at

Table 3. Average percentage of chalcid infested seeds in perennial Medicago species tested. Based on four plants in each of four replications.

Rank		Accession Number[1]	Mean Percentage Infestation	Least significant ranges[a] at the 1% level Duncan's Multiple Range Test
1.	falcata	115	41.0	1 - 57
2.	falcata	129	39.3	1 - 57
3.	lavrenkoi	255	34.6	3 - 63
4.	falcata	128	34.2	4 - 64
5.	falcata	137	34.1	4 - 64
6.	falcata	126	34.1	6 - 69
7.	falcata	130	32.5	7 - 70
8.	falcata	116	32.3	7 - 70
9.	falcata	138	31.2	7 - 70
10.	glandulosa	1833	29.9	7 - 70
11.	falcata	139	29.3	11 - 71
12.	agropyretorum	33	29.1	12 - 72
13.	hemicycla	232	28.4	12 - 72
14.	falcata	124	28.3	12 - 72
15.	glutinosa	84	28.2	12 - 72
16.	falcata	118	27.6	16 - 73
17.	prostrata	465	27.3	17 - 74
18.	hemicycla	233	27.1	18 - 77
19.	falcata	131	26.9	18 - 77
20.	falcata	122	25.6	18 - 77
21.	falcata	1845	25.4	18 - 77
22.	karatschaica	243	25.1	18 - 77
23.	glomerata	1529	24.7	18 - 77
24.	sativa	1838	23.9	18 - 77
25.	sativa	506	23.6	18 - 77
26.	falcata	120	23.5	18 - 77
27.	hemicycla	231	23.5	18 - 77
28.	falcata	1830	23.2	18 - 77
29.	sativa	507	23.1	18 - 77
30.	falcata	119	23.0	18 - 77
31.	agropyretorum	34	22.6	18 - 77
32.	falcata	121	22.2	18 - 77
33.	sativa	508	21.8	18 - 77
34.	prostrata	1682	20.8	18 - 77
35.	falcata	145	20.5	18 - 77

Table 3 continued .....

36.	falcata	117	20.4	18 - 77
37.	sativa	505	20.4	18 - 77
38.	falcata	125	20.2	18 - 77
39.	sativa	hex-sativa	19.6	18 - 77
40.	falcata	136	19.3	18 - 77
41.	falcata	1832	18.9	18 - 77
42.	falcata	134	18.8	18 - 77
43.	(hybrid)	can x hex-sativa	18.7	18 - 77
44.	falcata	123	18.4	18 - 77
45.	falcata	133	17.8	18 - 77
46.	sativa	1839	17.4	18 - 77
47.	sativa	1840	17.4	18 - 77
48.	falcata	135	17.3	18 - 77
49.	romenica	497	16.7	18 - 77
50.	sativa	504	16.5	18 - 77
51.	papillosa	890	16.5	18 - 77
52.	glandulosa	80	15.7	18 - 77
53.	hemicycla	235	15.3	18 - 77
54.	falcata	127	15.0	18 - 77
55.	falcata	132	14.4	18 - 77
56.	glandulosa	81	14.2	18 - 77
57.	rhodopaea	493	13.7	18 - 77
58.	hemicycla	234	12.0	58 - 89
59.	dzhawakhetica	98*	11.9	58 - 89
60.	papillosa	1864	11.9	58 - 89
61.	glandulosa	79	11.7	58 - 89
62.	sativa	1841	11.3	58 - 89
63.	suffruticosa	1549	11.0	58 - 89
64.	hemicycla	230	9.5	58 - 89
65.	tenderiensis	556	9.0	58 - 89
66.	suffruticosa	1544	9.0	58 - 89
67.	dzhawakhetica	97*	8.0	58 - 89
68.	carstiensis	45*	7.7	58 - 89
69.	glandulosa	78	7.2	58 - 89
70.	hemicoerulea	236	7.1	58 - 89
71.	leocarpa	554	6.4	58 - 89
72.	coerulea	223a	6.1	58 - 89
73.	hemicoerulea	1846	5.1	58 - 89
74.	carstiensis	44*	4.6	58 - 89
75.	saxatilis	586	4.2	58 - 89

Table 3 continued .....

76.	cancellata	43	4.2	58 - 89
77.	daghestanica	67	4.2	58 - 89
78.	coerulea	213	0.0	58 - 89
79.	coerulea	214	0.0	58 - 89
80.	coerulea	215	0.0	58 - 89
81.	coerulea	216	0.0	58 - 89
82.	coerulea	217	0.0	58 - 89
83.	coerulea	220	0.0	58 - 89
84.	coerulea	221	0.0	58 - 89
85.	coerulea	222	0.0	58 - 89
86.	coerulea	223	0.0	58 - 89
87.	sativa	509	0.0	58 - 89
88.	vardanis	585	0.0	58 - 89
89.	marina	1641	0.0	58 - 89
	20 DRC (control)		32.5 (average of 24 trials)	

$\overline{X} = 21.5$

F value for strains 6.70**

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[a] - a significant difference exists between any two means not found in the same range.

[1] - accession number refers to the Medicago collection at the Department of Genetics, University of Alberta, Edmonton, Alberta

* - indicates entries which were tested in the field

** - significant at the 1% level of probability

the 5 percent level of probability, but Medicago falcata strains were not significantly different.

Tables 4, 5, 6, and 7 show the ranked means of infestation among the strains of Medicago glandulosa, Medicago hemicycla, Medicago sativa and Medicago falcata, respectively.

Medicago Coerulea Species Study

Medicago coerulea was not infested in the free choice cages (see Table 3). These results were unexpected because Medicago coerulea has characters similar to Medicago sativa that was infested. To determine if Medicago coerulea was actually immune to the chalcid or merely an escape, two plants of Medicago coerulea were caged with a control plant (20 DRC). The control plant was infested, Medicago coerulea was not. However, the seeds of Medicago coerulea were infested when chalcids were caged on its racemes without access to a control plant. Consequently, the following experiment was conducted.

Several racemes from each of 5 plants of each strain of Medicago coerulea were tripped and the resulting pods exposed to chalcids in individual raceme cages. Control plants (20 DRC) were tripped and caged for each trial.

Each trial consisted of caging one female chalcid in a vial on a single raceme of Medicago coerulea consisting of at least three pods, and caging two female chalcids in a vial which contained one raceme from Medicago coerulea and one raceme from 20 DRC.

Table 4. Ranked means of infestation in strains of Medicago glandulosa.

Rank	Accession No.	Mean percentage infestation	Least significant ranges[a] 1% level of probability Duncan's Multiple Range Test
1	1833	29.8	
2	80	15.7	
3	81	14.2	
4	79	11.7	
5	78	7.2	

$\overline{X}$ = 15.6
F value for strains 9.46*

[a] Significant difference exists between any two means not found in same range
* Significant at the 1% level of probability.

Table 5. Ranked means of infestation in strains of Medicago hemicycla.

Rank	Accession No.	Mean percentage infestation	Least significant ranges[a] 5% level of probability Duncan's Multiple Range Test
1	232	28.3	
2	233	27.0	
3	231	23.4	
4	235	15.2	
5	234	12.0	
6	230	9.4	

$\overline{X}$ = 19.3
F value for strains 2.95*

[a] Significant difference exists between any two means not found in the same range
* Significant at the 5% level of probability.

Table 6. Ranked means of infestation in strains of Medicago sativa.

Rank	Accession No.	Mean percentage infestation	Least significant ranges[a] 5% level of probability Duncan's Multiple Range Test
1	1838	23.9	
2	506	23.6	
3	507	23.1	
4	508	21.8	
5	505	20.3	
6	1840	17.4	
7	1839	17.4	
8	504	16.4	
9	1841	11.3	
10	509	0.0	

$\bar{X}$ = 17.5
F value for strains 2.54*

[a] Significant difference exists between any two means not found in the same range
* Significant at 5% level of probability

Table 7. Ranked means of infestation in strains of Medicago falcata.

Rank	Accession No.	Mean percentage infestation	Least significant ranges[a] 5% level of probability Duncan's Multiple Range Test
1	115	41.0	
2	129	39.3	
3	128	34.2	
4	137	34.1	
5	126	34.1	
6	130	32.5	
7	116	32.3	
8	138	31.2	
9	139	29.3	
10	124	28.3	
11	118	27.6	
12	131	26.9	
13	122	25.6	
14	1845	25.4	
15	120	23.5	
16	1830	23.2	
17	119	23.0	
18	121	22.2	
19	145	20.5	
20	117	20.4	
21	125	20.2	
22	136	19.3	
23	1832	18.9	
24	134	18.8	
25	123	18.4	
26	133	17.8	
27	135	17.3	
28	127	15.0	
29	132	14.4	

$\overline{X}$ = 25.3
F value for strains 1.21 N.S.

N.S. Not significant

After ten days the vials were removed and the pods collected and analysed for infestation.

Table 8 shows the ranked means of infestation in the nine strains of Medicago coerulea.

Table 8. Ranked means of infestation in strains of Medicago coerulea.

Rank	Accession No.	Mean percentage infestation	Least significant ranges[a] 5% level of probability Duncan's Multiple Range Test
1	223	30.7	
2	221	25.6	
3	213	23.4	
4	222	21.8	
5	215	20.7	
6	214	19.7	
7	216	18.5	
8	220	14.9	
9	217	11.8	
Control (20 DRC)		36.3 (average of 69 trials)	

$\bar{X}$ = 20.8
F value for strains 2.25*
F value for replications 4.04**

[a] Significant difference exists between any two means not found in the same range.

* Significant at the 5% level of probability

** Significant at the 1% level of probability.

The data (see Appendix 5) shows the chalcids infested seeds of Medicago coerulea when caged on racemes of Medicago coerulea alone, but at a reduced rate. In the vials with both Medicago coerulea and control the chalcids infested the control at much higher rates than Medicago coerulea, sometimes infesting only the control.

Infestation of Annual Medicago Species

Several of the species had been previously reported as infestible (Sorenson and Knowlton, 1951; Bacon et al., 1964); the most frequently reported were Medicago arabica, Medicago polymorpha, and Medicago minima.

Large 12 inch individual pot cages were used at first, but only the control plants were infested. A few smaller 5 inch pot cages were tried, using the above mentioned species, but again only the controls were infested. Large free choice cages with controls included were also unsuccessfully tried, using the same three species mentioned above.

Individual raceme cages, which had proven successful in some preliminary studies on perennials, were tried on the annuals and infestation was obtained. This method of confining chalcids on the racemes proved effective in the following experiments.

Seeds from all 35 species were germinated and planted in small pots. Four pots of each species were included. When the

plants had matured until some of the pods were at a stage just prior to what was judged to be the infestible stage, chalcids were introduced to the raceme in individual raceme cages (vials). Since the species matured at different rates, some of them were infestible for a longer time than others, and to be sure to catch the susceptible stage of pod development, the vials were inspected daily and dead chalcids were replaced to maintain the raceme's exposure to the chalcid. Plants in each pot (4 per species) were tested.

When it was obvious that the pods were hard and maturing they were picked off, allowed to dry for a few days, shelled, and the percentage of seed infested was determined.

Table 9 shows the species that were infested and their average infestation over four replications. Twenty-three of the species were not infested. The twelve species that were infested ranged in infestation from 7.8 percent to 38.4 percent.

Analysis of variance showed differences are significant at the 1 percent level of probability if all species are considered, and are also significant between infested species.

Perennial Crosses

The data from the original tests on the perennials (see Table 3) indicated there was considerable variation in infestation among species. Medicago coerulea, which has characteristics

Table 9. Average percentage infestation in annual Medicago species infested. Based on one plant in each of four replications.

Rank	Species	Accession No.	Percentage infestation	Least significant ranges[a] 1% level of probability Duncan's Multiple Range Test
1.	polymorpha	418	38.4	
2.	tornata	563	36.7	
3.	arabica	19	22.6	
4.	praecox	1306	20.0	
5.	shepardi	684	20.0	
6.	tenoriana	1499	19.5	
7.	minima	324	17.0	
8.	coronata	61	14.8	
9.	rugosa	795	14.0	
10.	orbicularis	370	10.9	
11.	secundiflora	553	10.5	
12.	lupulina	294	7.8	

$\bar{X} = 19.4$
F value for species 2.92*
F value for replications 1.36 N.S.

[a] A significant difference exists between any two means not found in the same range.

* Significant at the 1% level of probability

N.S. Not significant

essentially the same as diploid Medicago sativa, was not infested in the free choice cages, whereas diploid Medicago sativa was. Medicago falcata was also heavily infested and it has many characteristics similar to the above two species.

In view of some earlier work (1963) by the author on pod characteristics in relation to infestation, that showed a consistent variation in infestation with variations in pod morphology, it was decided to cross species having different pod characters and determine if the difference in infestation was due to pod shape.

A plant from Medicago coerulea (217-1-1), which produced a large amount of seed, and which was not infested in the free choice cages, was selected as the male parent for four crosses. It had pods with nearly three full tightly coiled whorls.

Four species were selected to cross with 217-1-1 (Table 10). These had relatively high infestation in the free choice cages. Three had "open" pods, i.e., pods with sufficient space between whorls during the optimum infestation period of development (9 days after tripping) to allow a female chalcid in between them to oviposit. The space required was estimated to be 1.0 to 2.0 mm. These three "open" pod species were: Medicago falcata (125-1-4) 0 to $\frac{1}{2}$ turns per pod; Medicago prostrata (1682-2-4) 1 to $1\frac{1}{2}$ turns per pod; and Medicago glomerata (1529-3-4) $\frac{1}{4}$ to $\frac{3}{4}$ turns per pod. One tightly coiled species was used to cross

with 217-1-1. It was a diploid Medicago sativa strain called Saskatchewan White, having 1 to 1½ turns per pod with the whorls close together (< 1.0 mm).

Table 10. Summary of characters of parents in four perennial crosses.

	Parent plants				
	Male parent	Female parents			
Character	217	1682	1529	125	Sask.
Pod shape	tight	open	open	open	tight
Turns per pod	3	1 - 1½	¼ - ¾	0 - ½	1 - 1½
Flower color	purple	yellow	yellow	yellow	white

Crossing was effected with the aid of tweezers, needle nose scissors and a suction apparatus. The standard petals were cut off, the keel petal opened, and the pollen sucked away, after momentarily dipping the emasculated flower in 57 percent ethanol and water and then tap water.

Pollen was collected from the male parent, which in all cases was Medicago coerulea (217-1-1), by opening the flowers with a toothpick as previously described, and touching the pollen to the exposed stigma of the female parent.

The resulting seed from the four crosses was germinated in petri dishes as previously described and planted in flats. The F_1 plants were fertilized among themselves for each cross,

and the resulting seed was germinated, planted as before, and grown to maturity in the growth chamber.

When the F_2 plants were in bloom they were pollinated among themselves with toothpicks, placed in free choice cages, and insects were introduced at the optimum ovipositional stage of pod development (9 days). The insects were allowed to oviposit over a 13 day period, the pods were collected separately from each plant, and analysed according to pod characteristics and infestation. Table 11 shows the characters of the F_1 plants; Tables 12 and 13 show the distribution of the F_2 plants for tight versus open pods and flower color.

Table 14 shows the distribution of the F_2 plants from the four crosses when classified according to the number of curls in the pods. Table 15 shows the average percentage of seeds infested for the four crosses when arranged according to the number of curls in the pods.

Appendices 4a, b, c, d, show that the chalcids had access to a wide range of different pod characteristics from each cross. The cross 217 X Saskatchewan White had all "tight" pods, but otherwise there were both "open" and "tight" pods, and a variety of pods, with regard to number of curls per pod, from each cross. Table 16 shows the relative infestation of seeds in pods that were "open" or "tight". The "open" pods had a much higher average infestation than the tight ones.

Table 11. Summary of characters of F_1 plants in four perennial crosses.

Character	Cross 217 X 1682	217 X 1529	217 X 125	217 X Sask
Pod shape	tight	tight	open	tight
Turns per pod	1 - 1½	1 - 1½	½ - 1	1 - 1½
Flower color	green to purple variegated	purple to green variegated	green to yellow variegated	purple

Table 12. Distribution of F_2 plants in four crosses classified according to tight versus open pods.

Character	Cross 217 X 1682	217 X 1529	217 X 125	217 X Sask
Tight	31	25	23	61
Open	13	22	25	0

Table 13. Distribution of F_2 plants in four crosses classified according to flower color.

Character	Cross 217 X 1682	217 X 1529	217 X 125	217 X Sask
Purple-green			22	
Green-yellow	9	6		
Dark purple	12		15	44
Light purple	11	16	11	
Yellow	12	25		
White				17

Table 14. Distribution of F_2 plants in four crosses arranged according to number of curls per pod.

No. of curls per pod	Cross 217 X 1682	217 X 1529	217 X 125	217 X Sask.
0.0 - 0.5	0	0	8	0
0.5 - 1.0	0	3	17	0
1.0 - 1.5	7	17	16	23
1.5 - 2.0	18	15	7	16
2.0 - 2.5	14	10	0	13
2.5 - 3.0	5	2	0	9
Total plants	44	47	48	61

Table 15. Percentage infested seed in four crosses arranged according to number of curls per pod.

No. of curls per pod	Cross 217 X 1682	217 X 1529	217 X 125	217 X Sask	Average
0.0 - 0.5	-	-	30.1	-	30.1
0.5 - 1.5	-	20.6	20.9	-	20.8
1.0 - 1.5	23.6	17.4	19.8	17.1	19.5
1.5 - 2.0	16.2	15.9	14.6	10.0	14.2
2.0 - 2.5	9.8	8.4	-	6.5	8.2
2.5 - 3.0	8.5	6.5	-	4.1	6.4
Average per cross	14.4	14.8	21.2	11.1	

Table 16. Percentage infested seed in four crosses arranged according to tightness of curls in the pods.

Pod Class	Cross 217 X 1682	217 X 1529	217 X 125	217 X Sask	Average
Open	23.1	19.9	23.8	-	22.3
Tight	11.6	10.2	18.2	11.1	12.8

Figures 1, 2, 3, 4, are graphs showing regression of infestation on numbers of curls per pod for each of the crosses. Comparisons of regression coefficients were made in all possible combinations by the use of a homogeneity of regression test (Steele and Torrie, 1959). Table 17 lists the crosses, the regression coefficients, and the calculated value for each. Only two of the comparisons showed significantly different coefficients, both involving 1682 (Medicago prostrata). The comparisons indicate that the slopes of the various regression lines are very similar.

Table 17. Summary of tests of regression coefficients for homogeneity among four crosses.

Comparison	Regression coefficients		Calculated t value	Degrees of freedom
Sask X 217 - 1682 X 217	- 9.1	-11.4	2.49*	101
Sask X 217 - 1529 X 217	- 9.1	- 7.8	1.30 N.S.	104
Sask X 217 - 125 X 217	- 9.1	- 8.8	0.20 N.S.	106
1682 X 217 - 1529 X 217	-11.4	- 7.8	3.27**	97
1682 X 217 - 125 X 217	-11.4	- 8.8	1.57 N.S.	98
1529 X 217 - 125 X 217	- 7.8	- 8.8	0.58 N.S,	91

* Significant at the 5% level of probability
** Significant at the 1% level of probability
N.S. Not significant

Insect Biotype Determination

Some difficulty was experienced in obtaining any infestation, in the original single pot cages, on annuals that had previously been reported as infestible (Sorenson and Knowlton, 1951; Bacon et al., 1963). To determine whether or not there was a different

Figure 1. Graph of the regression of infestation on number of curls per pod for the cross Sask. X 217.

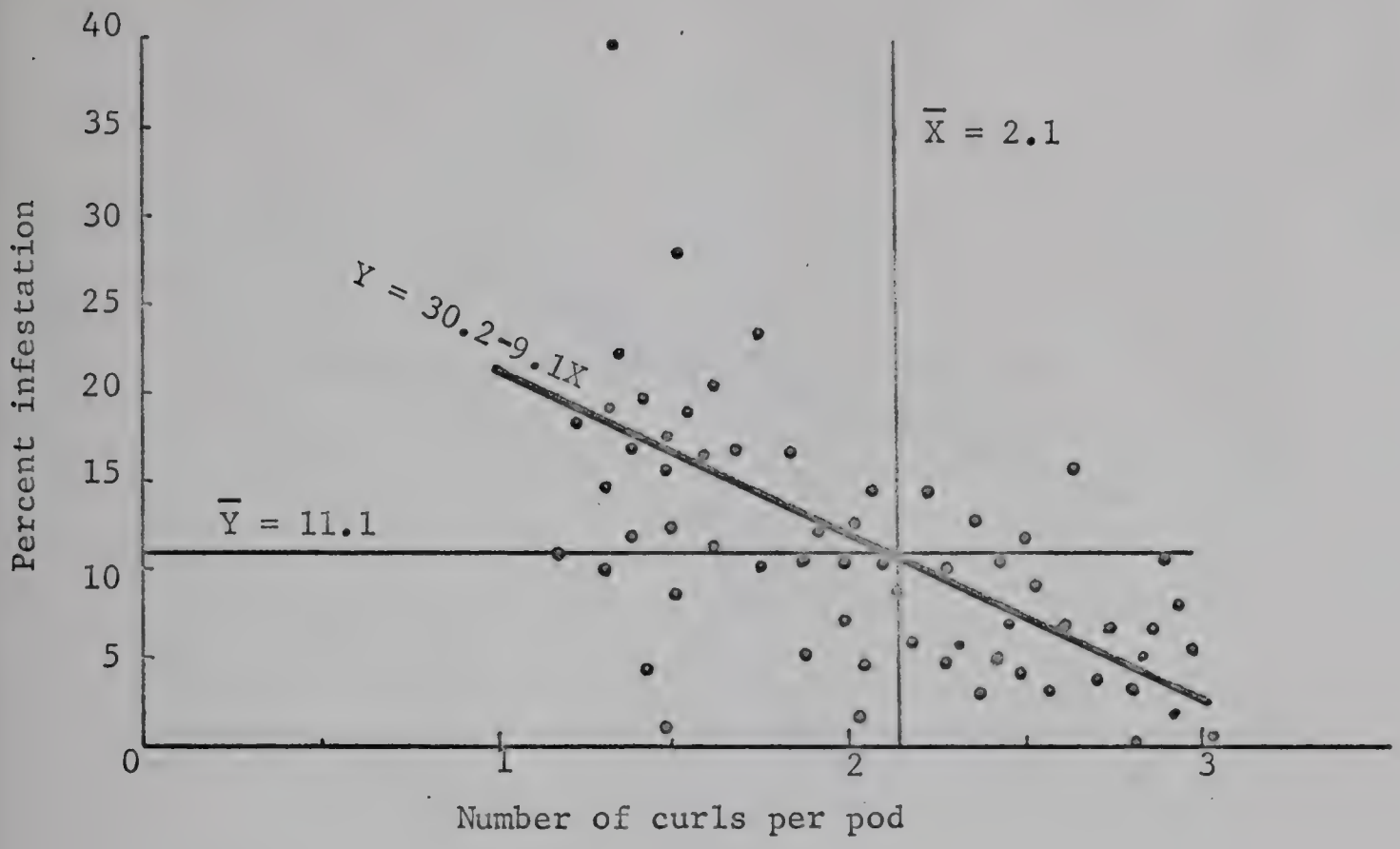


Figure 2. Graph of the regression of infestation on number of curls per pod for the cross 1682 X 217.

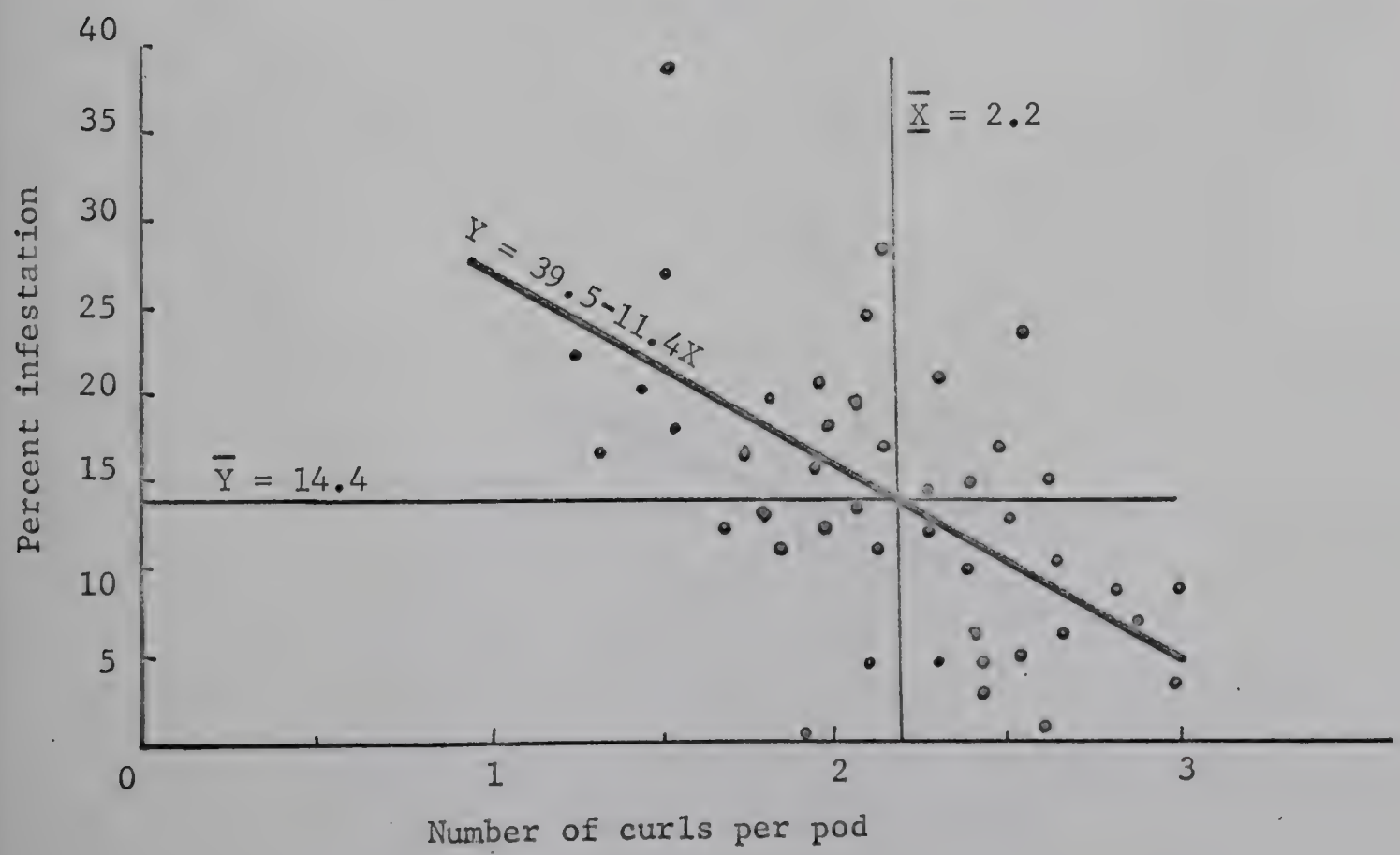


Figure 3. Graph of the regression of infestation on number of curls per pod for the cross 125 X 217.

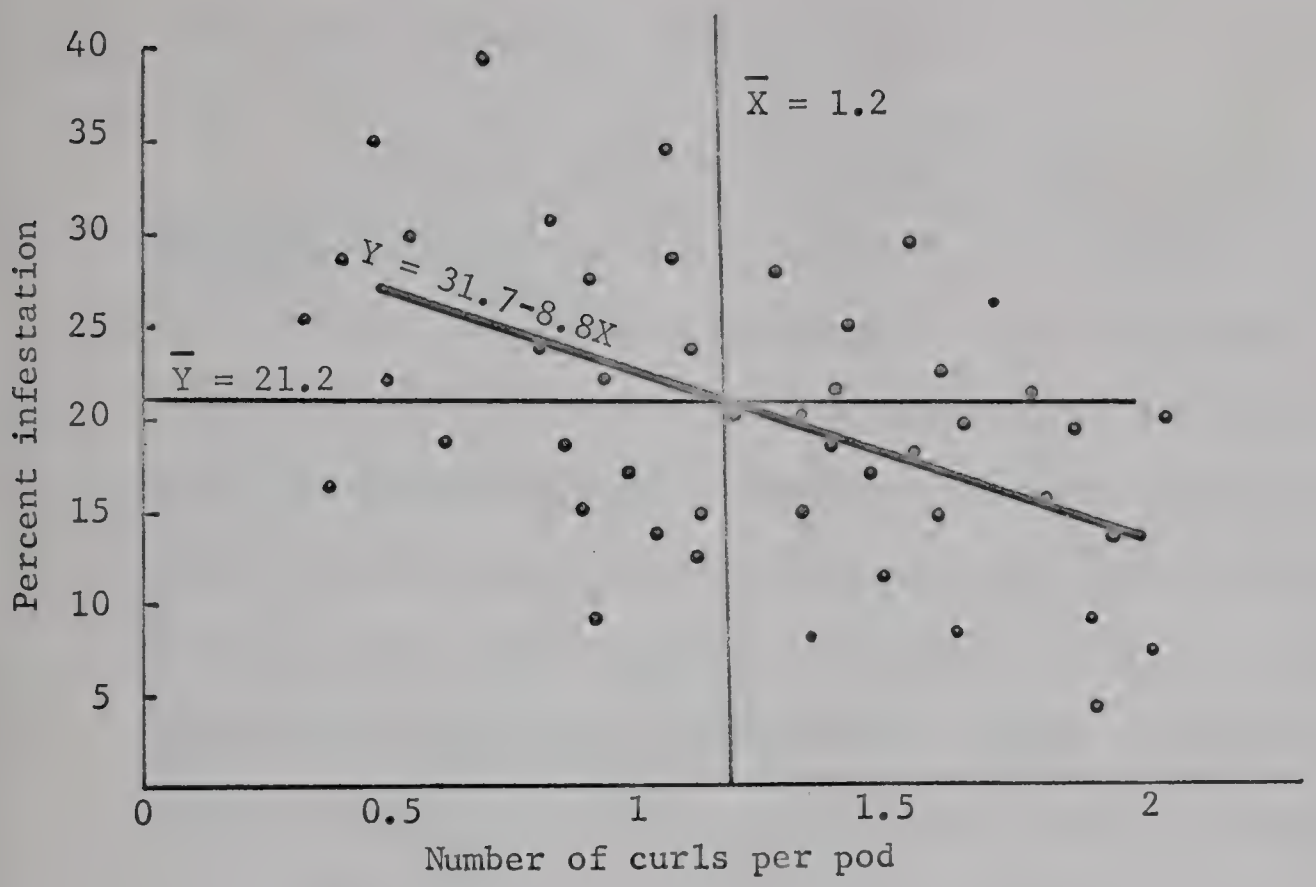
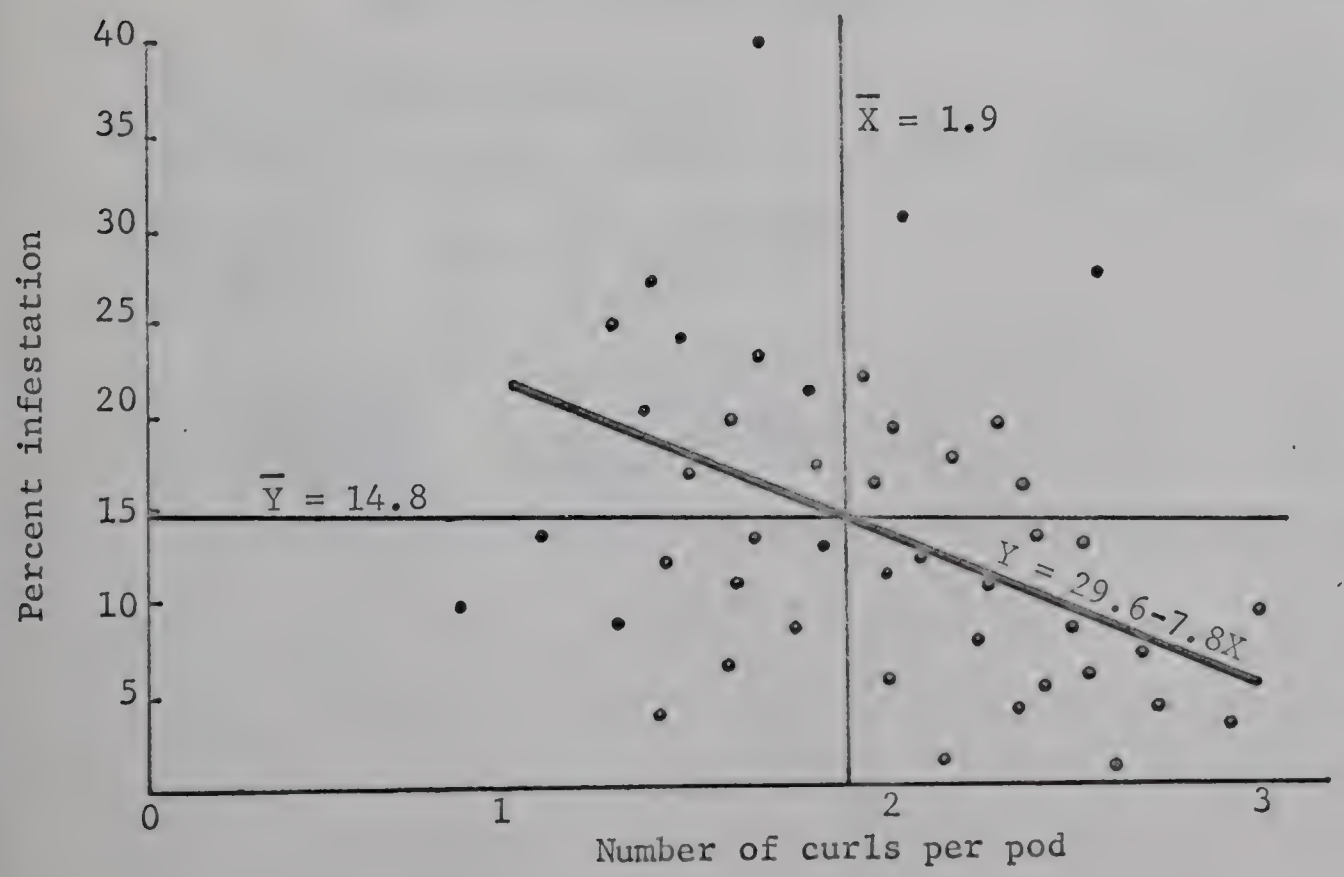


Figure 4. Graph of the regression of infestation on number of curls per pod for the cross 1529 X 217.



biotype infesting alfalfa, which was the source of these insects, and bur clover, which should have been infested, a crossing experiment using a common host was conducted.

Tests that followed the original tests on the annuals showed Medicago tornata to be easily infested, and this species was selected as a host for the crossing experiment. To obtain virgin females, individual infested seeds from both alfalfa and bur clover were placed in emergence cages. When the individual females emerged a male from the opposite source was caged with her in a vial on a suitable raceme and ovippsition allowed to occur. In four cases females were caged on a raceme without a male. Table 18 shows the manner in which the crosses were made and the resulting progeny, which were counted and sexed as they emerged.

Table 18. Results of cross breeding tests between chalcids reared from Medicago polymorpha and Medicago sativa. Medicago tornata was used as a host plant.

Parents (from)		No.	Progeny			
Female	Male	trials	Female	Male	Total	
M. sativa	M. polymorpha	2	17	14	31	
M. sativa	M. sativa	1	7	8	15	67
M. sativa	None	2	0	21	21	
M. polymorpha	M. sativa	2	26	19	45	
M. polymorpha	M. polymorpha	1	15	11	26	76
M. polymorpha	None	2	0	25	25	

The results indicate that there was probably no difference in biotype between the insects from alfalfa and bur clover (Table 18). The females coming from bur clover had a greater number of offspring than those coming from alfalfa, but those coming from alfalfa had been in diapause for two years and had undoubtedly lost some of their vigor.

Unmated females still laid eggs that developed but produced only males; the total number of offspring in these cases was reduced 20 to 30 percent. These results agree with a similar experiment by Batiste (1964) and indicate that the sex determining mechanism in Bruchophagus roddi is of the arrhenotokous type common in hymenoptera, wherein males are haploid and females diploid and unfertilized eggs develop parthenogenetically into males.

Insect Source Versus Choice of Host

Because of difficulty in obtaining infestation on annuals in the preliminary experiments, a test was set up to determine whether the source of the insects affected their infestation in other host species.

Chalcid from bur clover obtained from Arizona and Texas were allowed to emerge, mate and infest a series of racemes of different host species, i.e., 1) bur clover, (Medicago polymorpha), 2) alfalfa (20 DRC), and 3) bur clover and alfalfa. Chalcids from alfalfa obtained from California were allowed to emerge, mate and infest the same series. Two trials of each combination were conducted.

Two females and one male were caged in vials which were placed on racemes of the respective host plants. Bur clover racemes that had two pods each and alfalfa racemes with five pods each were used in all cases. In the four vials that held both host plants, four females and two males were used to maintain the chalcid:pod ratio.

Table 19 shows the results of this experiment with the totals of the two trials combined. Chalcids from bur clover infested 34 of 146 seeds they were exposed to. Chalcids from alfalfa infested 28 of 145 seeds. When chalcids from either source were placed on either host separately they oviposited in the seeds at approximately the same rate. However, when the chalcids were exposed to both host species at the same time the chalcids laid three to four times as many eggs in alfalfa seeds as they did in the bur clover seeds.

Table 19. Infestation results in chalcid source versus host choice study.

	Insect Source					
	Alfalfa			Bur clover		
Insects placed on	Total seeds	Infested seeds	%	Total seeds	Infested seeds	%
Alfalfa only	58	6	10.3	45	8	17.8
Bur clover only	20	8	40.0	23	7	30.4
Alfalfa and bur clover						
Alfalfa	46	11	23.9	53	15	28.3
Bur clover	21	3	14.3	25	4	16.0

Seed Weight Versus Infestation

In the original tests on the perennials the chalcids seemed to favor the species with larger seeds. Some very large seeded ones were not infested, i.e., Medicago marina and some small seeded ones were, i.e., diploid Medicago sativa, but in general it appeared that the smaller seeded species were less infested than the larger seeded ones.

To obtain further information on this hypothesis the seed weight of all the entries was determined and this data was correlated with infestation data obtained in the free choice cages. Four lots of 100 seeds from each entry were weighed on a mono-pan balance and the average taken. The equivalent weight of 1,000 seeds was then calculated and these data were correlated with the infestation data.

The seeds ranged in weight from 0.5 grams per 1,000 seeds for Medicago sativa (Accession no. 509) to 3.29 grams per 1,000 seeds for Medicago marina (Accession no. 1641), but the majority had seeds weighing from 1.0 to 2.0 grams per 1,000 seeds.

The seed weight and percentage infested seed showed a high degree of correlation. When all the entries were considered the correlation coefficient was calculated to be $r = .611$. When all of the uninfested entries were excluded from the analysis the correlation coefficient was calculated to be $r = .844$.

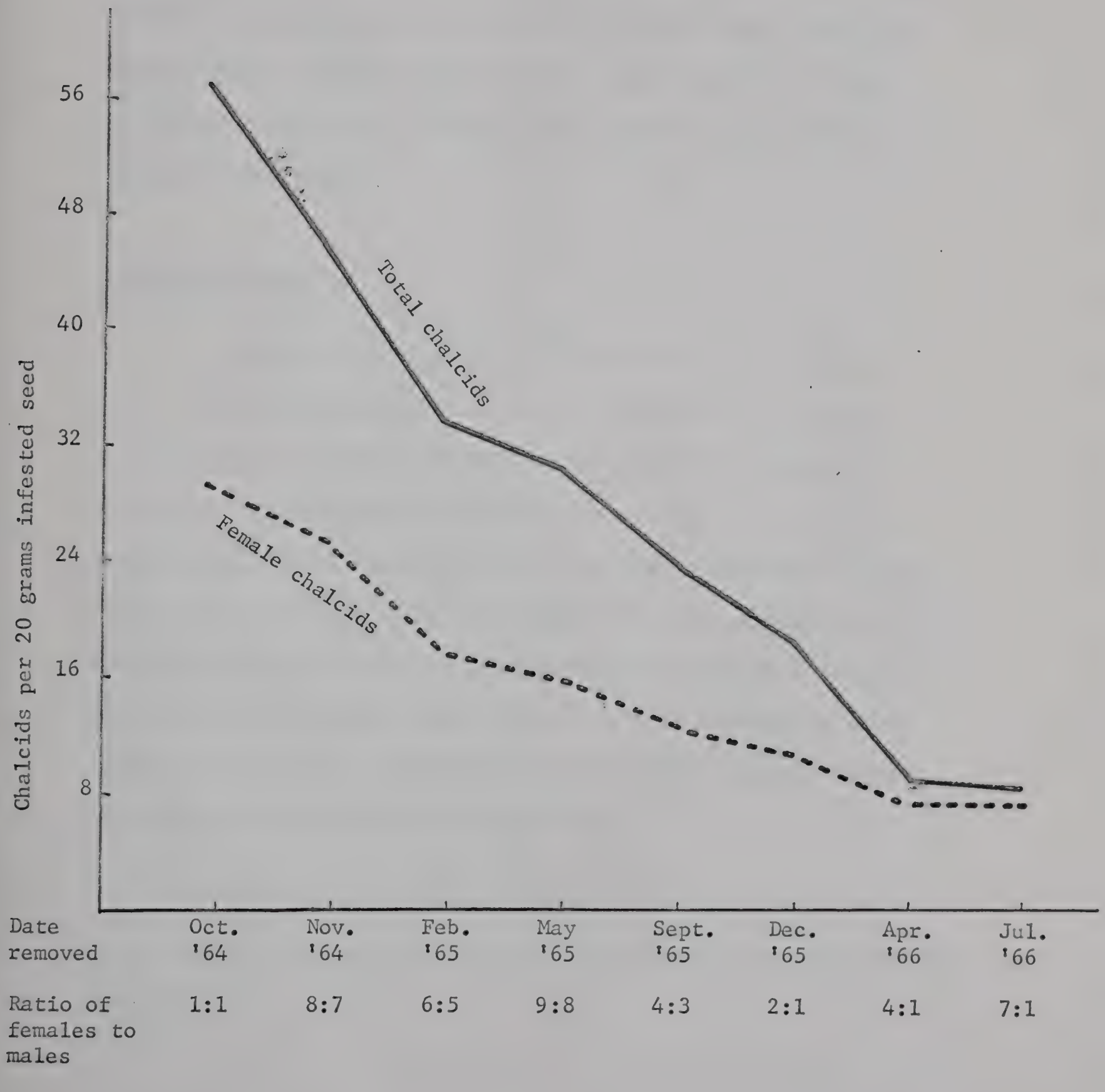
Chalcid Emergence From Stored Seed

Emergence records were kept on the chalcids developing from the infested seed received from Davis, California. The seed was heavily infested, and had been in cold storage at 43° F. for twelve months when received. Five 20 gm. samples of seed were removed from storage at periodic intervals and numbers of chalcids and their sex were determined. Figure 5 shows the dates seed was removed from storage and the resulting chalcids.

Total numbers emerging decreased almost continuously throughout the two year period, but females had less mortality than males. The male to female ratio remained close to 1:1 during the first twelve months of storage in our cold rooms (24 months total cold storage) but during the second year in our rooms the ratio declined to about 7 females to 1 male emerging. Counts were not taken after July 1, 1966, as the remainder of the seed was set out in the field to supplement natural populations in a field test.

It was noted that females emerging after 2 years in diapause laid fewer eggs than those emerging after a shorter time in diapause. Insects that had been in diapause only two months laid 15 and 21 percent more eggs in the two experiments involving chalcids from different sources (see choice of host and biotype studies) than those that had been in diapause for more than two years. This could have been due to exhaustion of body fat over the prolonged hibernation period, and consequent resorbtion of eggs; a starvation effect with a drop in fertility or longevity or both.

Figure 5. Emergence of alfalfa seed chalcid from 1963 seed showing numbers and sex ratio.



DISCUSSION

The investigations conducted in this study indicate that infestation of Medicago species by Bruchophagus roddi depends on a number of factors. A list of influencing factors is as follows: 1) Pod characteristics such as curls, tightness, seed position, and hairyness; 2) Seed size; 3) Physiological age of the seed; 4) Climatic conditions; 5) Host plant. These are not listed in any particular order.

Pod Characteristics

The number of curls in a pod affected the infestation rate. This was demonstrated by the data from the four crosses of species with varying pod curls. The correlation between a low number of curls and a high infestation rate was shown to be significantly high. Analysis of the data using regression showed that most of the regression coefficients were not significantly different, implying that the insects were not influenced in their activity by which plant they were on, but by the number of curls in the pod that they were ovipositing in. Pods with high numbers of curls were consistently less infested.

The tightness of the pod between the curls also affected infestation. The tighter the curls in the pod the less infestation there was.

The most probable explanation of the situation described above is one of physical accessibility of the seed in the pod. The female chalcid locates a seed within a pod by tapping with her antennae, but there is almost invariably a swelling in the pod coat where she oviposits. If a pod is too tightly coiled the chalcid cannot orient herself into a position that will allow penetration of the pod and seed coat. It would appear that the chalcid seeks a pod that has relatively straight surfaces, i.e., sickel shaped pods, to deposit eggs in. Perhaps a curved surface is more difficult to penetrate, as the female must bend her abdomen almost at right angles to the thorax and head, and the amount of bending required would greater on a curved surface than on a straight one.

The position of the seed in the pod affected its availability for oviposition. When a pod had a number of tightly coiled curls, and the seed was located very close to the ventral suture, there was little chance of infestation. This was illustrated by the two species belonging to the group (Anonymous, 1950) Orbiculares. Medicago carstiensis, a perennial, and Medicago orbicularis, an annual, both had pods that were large and open enough in the early stages to allow the chalcids in between curls, but the relatively small seeds were located very close to the ventral suture. In Medicago carstiensis there were no seeds infested except those at the ends of the pods where the insect had access to them.

In a tightly coiled pod like that of Medicago coerulea there would be even more difficulty in oviposition because the pod end sometimes has no seed and acts as a shield for those further down in the pod.

Hairyness of pods at the optimum ovipositional time seemed to affect infestation. There were several species with hairy pods: Medicago lanigera, Medicago marina, Medicago papillosa, and several Medicago falcata strains. The first two were not infested, but the latter two were. Examination of the pods at 8 to 12 days after tripping showed that hairs were not formed extensively on Medicago papillosa and Medicago falcata, but that they had formed on the uninfested ones. Medicago lanigera was especially "woolly".

Seed Size

The seed size versus infestation correlation data indicated that there was a positive correlation between the size of seed and infestation. This could have been a result of oviposition difficulty in small seeds, failure on the part of the female chalcid to locate small seeds, or the small seeds may give lesser stimulation to oviposition. Probably all these factors affected infestation, and no attempt was made to measure the separate effects.

Physiological Age of Seed

Batiste (1964) studied the age of seeds of Lotus tenuis infested by Bruchophagus kolobovae, and Strong (1962) reported on

ovipositional activity of Bruchophagus roddi in relation to age of the seeds. Both investigators reported that infestation was confined to a rather rigid time period. Batiste (1964) reported that many seeds probed by the insects contained no egg. Apparently the chalcids recognized suitable seeds by penetrating them with their ovipositor. If the seed was not at the right stage no egg was laid even though the seed itself was penetrated.

In this study the same phenomenon was observed. In the infestation of the annuals when the seeds were dissected after being exposed to the chalcid, many seeds were observed that had probe scars (sometimes several scars in one seed), yet contained no egg or larvae. Sorenson (1934) reported only a small percentage of the seeds he observed contained a chalcid that failed to develop completely before the seed hardened. This would indicate that female chalcids are "careful" to oviposit in seeds that will allow development of their offspring.

The number of days from tripping to the optimum ovipositional stage varied with the plant and growing conditions. The pods of the control plant (20 DRC), used in most of the studies reported in this thesis, were often ready for oviposition in six days, whereas several of the perennial species' pods were not ready at thirteen days. The annual species exhibited great variation in pod development and many times it was difficult to "catch" the pods at the right time. Under normal growing conditions pods were at the optimum in Medicago sativa at nine days.

Host Plants

Virtually all of the perennial Medicago species were infested. It is interesting to note that all but two of the perennial species infested belong to the section Falcago (Anonymous, 1950). The only species in the section available for testing that were not infested were Medicago vardanis and Medicago marina. (Medicago arborea belongs to this group but did not blossom). Medicago vardanis has characteristics similar to those of Medicago falcata. The pods of Medicago vardanis are curled but not tightly and there is no obvious reason why it was not infested. Medicago marina has pods that are large but are curled and tight, and quite hairy at the ovipositional stage.

The two species not belonging to the section Falcago that were infested were Medicago carstiensis and Medicago daghestanica. Medicago carstiensis belongs to the section Orbiculares, also containing the annual species Medicago orbicularis which was infested. Medicago daghestanica belongs to the section Euspirocarpae containing Medicago arabica, Medicago polymorpha, and Medicago praecox, which were all infested.

It is significant that two of the sections outlined (Anonymous, 1950) had no infestation in any of their species, i.e., Intertextae, and Rotatae. There were several sections in which the species were nearly all infested, i.e., Lupularia, Orbiculares, Euspirocarpae, and Falcago. The other sections, Scutellatae, Pachyspirae and Leptospirae had some species infested, others not.

These observations are not surprising, however, when one considers that pod characteristics were a major criterion in classifying Medicago species originally.

Insect Choice

Almost all of the perennial species were infested in the free choice cages. The notable exception to this was Medicago coerulea. A review of the original data showed that there was always a pot from one of the strains of Medicago falcata in the cage along with Medicago coerulea and of course the control plant. The insect could "choose" between Medicago falcata and Medicago coerulea or any other species such as Medicago sativa (509). Characters were not as "desirable" in Medicago coerulea as in Medicago falcata or the control plant. When the chalcids had only Medicago coerulea in which to oviposit, they did so, but at a reduced rate. The reduction in infestation could be due to physical inaccessibility, or to the pods being non-preferred.

The annual species of Medicago were discriminated against by the chalcid in the experiments in this study. When the insects had a perennial control plant in which to lay eggs they did not oviposit in the annuals. When chalcids were confined to a single infestible species they oviposited in some of them, but in most cases at a reduced rate.

In the preliminary tests on the annuals using individual pot cages no infestation was obtained even though the insects had no access to a control plant. Two Medicago polymorpha plants and a control plant were caged with insects in a similar manner in the field to see if the type of cage affected oviposition. The weather

turned cold and heavy rains fell shortly after caging, and examination of the seeds showed no infestation in any of the plants. There seems to be no logical explanation for the results of these preliminary tests. However, infestation was obtained using individual raceme cages under conditions where no "choice" was given the chalcids.

Climatic Conditions

The alfalfa seed chalcid Bruchophagus roddi Guss. is not prevalent in this locality (Edmonton, Alberta). The chalcid is found here, but weather conditions are not conducive to its development. Long, cold winters probably destroy overwintering chalcids, and the rainfall and cool weather during the summer prevent population buildup. In addition there is very little alfalfa grown for seed here.

In the summer of 1964 the author selected pods from some Medicago falcata plants growing near a field that held seed alfalfa in 1963, and determined the infestation rate. Of the seeds dissected, 16.4 percent contained a developing larva. Identifications were not made, but it was assumed these were Bruchophagus roddi.

All of the perennial plants from the original tests were transplanted into a nursery in the field at Parkland University Farm in 1965. It was hoped that chalcid populations as indicated by the 1963 estimations, would be sufficient to get an estimate of infestation under natural conditions here, but this proved impossible.

Periodic sweeps taken in the nursery showed chalcids virtually non-existant after a prolonged cold rainy period in early August, 1966, which was before the plants had set pods. The sweeps showed an increase in numbers only after July 1, 1966. The maximum number collected was 93 in 50 sweeps, using a standard 15 inch insect net, on July 22, 1966.

Similar sweeps taken in 1965 showed only a few more insects than those taken in 1966. Maximum number in 1965 was 132 in 50 sweeps, collected on July 22 using a standard 15 inch insect net.

The author (1963) reported that chalcid activity was greatly reduced when atmospheric temperatures went below 70° F. Chalcids were most active when temperatures were relatively high, i.e., above 85° F.

Other Factors

There are undoubtedly many factors involved in the infestation of Medicago species that have not been considered in this study. The biochemical aspects of the host:parasite relationship have been studied very little (Kamm and Fronk, 1964). Painters' (1958) "antibiosis" is no doubt a factor in Medicago resistance to the chalcid. Ecological conditions, i.e., host:parasite populations have not been studied extensively and could be a significant factor in infestation or resistance, and perhaps additional study of the biology of the chalcid would yield significant information on why the chalcids infest some hosts and not others and some at different rates than others.

Inheritance of Flower Color and Pod Characteristics

The results of the crosses between plants of different pod characters, as shown in Tables 11, 12 and 13, indicate that both flower color and pod shape are complex in their inheritance.

It would appear from the cross 217 X Sask (Table 13) that: 1) the character white flower color, or the absence of anthocyanins, was recessive to purple; 2) flower color was simply inherited and due to a single pair of genes. However the other three crosses yielded data for which no explanation could be given except that flower color in these crosses was controlled by several genes and was complex in its inheritance. This agrees essentially with the work done by Lesins (1956) and Twamley (1955) on flower color in alfalfa. There was no correlation between color of flower and infestation by chalcids in the F_2 plants.

Both the F_1 and F_2 individuals of the cross 217 X Sask. had tightly coiled pods suggesting the parent plants of this cross were homozygous at all loci for this character. The other three crosses did not fit any expected genetic ratio to give an explanation of inheritance of pod coiling.

The number of curls per pod seemed to be inherited quantitatively. The average number of curls per pod in the F_2 individuals fell between the averages of the parents for each cross.

Since susceptibility or resistance of a plant to the chalcid is partially associated with pod characters, further investigations

into the inheritance of pod characters could yield valuable information for breeding a resistant strain of alfalfa. In addition, back crossing of the resistant or susceptible F_2 individual from the crosses to the appropriate parent and testing of progeny with chalcids would indicate whether resistance or susceptibility could be significantly altered in succeeding generations in a breeding program.

Practical Application

One of the objectives of the research reported in this thesis was to see if a commercially useable forage resistant to infestation by Bruchophagus roddi could be developed from any resistant plant or plants tested in this study. Except for developing a synthetic variety, through selection of individual plants which are "more resistant" and using polycross breeding methods, there seems little practical use of the results of this investigation at present. Most of the plants tested were diploid and would not produce forage on an equal basis with the tetraploid alfalfas now being used. The most practical method at present seems to be selection for a synthetic variety among tetraploid varieties already in use, and this is being done (Nielsen, 1966).

Some of the annuals tested could produce good quality forage in economically practical amounts, but the necessity of reseeding each year precludes their use as a seed crop even though they are immune to the chalcid.

SUMMARY

Twenty-five species of Medicago perennials including 89 strains were tested for infestation or resistance to the alfalfa seed chalcid Bruchophagus roddi Guss. Almost all of the perennials were infested, but at significantly different rates.

Three strains of Medicago arborea and one strain of Medicago pironae were available but did not blossom well enough to test.

Differencesin infestation among strains within a species were demonstrated in four of five cases where there were a sufficient number of strains for a significant test.

Several accessions that appeared to be immune at first were later shown to be infestible.

A series of plant crosses between selected plants were made to determine what pod characteristics were attractive to the chalcids, and whether the insects were attracted to certain plants regardless of pod characteristics. The analysed results show that the insects, at least in these crosses, infested without regard to the plants the pods came from. Inheritance of these characters was shown to be genetically complex.

Thirty-five species of Medicago annuals were tested for infestation to the alfalfa seed chalcid Bruchophagus roddi Guss. Twelve of the annuals were infested, but at significantly different rates. Twenty-three annual species were not infested in these tests.

A cross between chalcids from different sources was made to determine whether chalcids infesting alfalfa were the same biotype as those infesting bur clover. Results of this experiment indicate that the two hosts are infested by the same biotype.

Chalcids from bur clover were placed on alfalfa for infestation, and vice versa, to determine whether the source of the insect affected the rate of infestation in a different host. Results showed that insects from either source will infest either host at approximately equal rates.

A correlation coefficient was calculated between infestation data and seed weight of the perennial entries. A relatively high positive correlation exists, indicating that the insects infested the larger seeds more readily.

An opportunity was available to study the effects of prolonged storage (diapause) on the alfalfa seed chalcid. Emergence data was kept on insects emerging from infested seed kept in cold storage. Results showed that mortality increases with time in storage, but after 24 months the mortality of males greatly exceeded that of the females.

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Appendix I. Data from infestation of perennial Medicago species in free choice cages. Seeds/infested seeds.

Acc. No.	Pot No.	Plant number 1	2	3	4	5
33	1	92/27	9/1	5/0		
	2	16/5	24/11	12/3		
	3	41/9	21/9	66/21	49/16	
	4	73/18	10/2	4/1	46/9	
34	1	30/5	12/3			
	2	5/1				
	3	79/16				
	4	65/20				
43	1	17/3	11/1	41/1	81/4	
	2	111/5	12/0	91/4	11/1	28/1
	3	46/2	9/0	16/1		
	4	25/0	9/0	5/0		
67	1	9/0	3/0	0/0	16/1	
	2	14/0				
	3	21/1	14/1			
	4	13/0	16/2			
78	1	51/3	114/6	165/6		
	2	23/0	20/0	31/2		
	3	38/3	130/10	72/6		
	4	26/4	21/4	13/0		
79	1	93/6	40/4	41/4	4/0	
	2	159/11	52/0	40/3	117/8	
	3	27/8	103/21			
	4	9/2	3/0	39/4		
80	1	44/9	63/12			
	2	43/11	36/8			
	3	4/2	87/11	13/2		
	4	10/0	39/4			
81	1	133/9	51/3	59/7	112/3	
	2	31/4	25/2	38/0	22/3	
	3	12/1	41/12	54/12		
	4	4/1	19/4	74/11	9/1	
1833	1	13/5	5/1			
	2	16/3	8/3			
	3	16/4				
	4	17/6	47/17			

Appendix I continued .....

Acc. No.	Pot No.	Plant number 1	2	3	4	5
84	1	12/4				
	2	7/2				
	3	3/0	6/0	26/1		
	4	38/18	2/0			
115	1	19/3	34/8			
	2	17/10				
	3	29/11	9/1			
	4	11/4	8/6			
116	1	49/11	39/5			
	2	1/9	9/8	12/5		
	3	96/12	41/4	11/1		
	4	55/22	63/20	47/13	16/10	
117	1	243/50				
	2	39/11	13/1			
	3	5/1	39/11	70/8		
	4	30/7	62/14	45/8	83/17	
118	1	121/51	27/12	181/43	46/6	
	2	7/0	38/4	8/1		
	3	54/15	40/12			
	4	57/26	96/30			
119	1	63/8	16/1	12/2	162/26	
	2	91/18	72/11	37/16	8/1	31/4
	3	78/10	47/10	8/3	7/1	
	4	43/17	3/1			
120	1	72/22	99/22	4/0		
	2	3/0	4/0			
	3	74/16				
	4	51/23	40/21	52/22		
121	1	29/4	50/10			
	2	4/o	36/10	29/9		
	3	102/11	29/13	57/18	20/3	
	4	18/2	53/10	32/10		
122	1	10/0	24/1	27/3	46/3	12/2
	2	16/3	27/8	12/0		
	3	31/13	2/0			
	4	18/6	12/9	99/17		
123	1	32/9	55/14			
	2	27/6	17/1	9/0	43/8	
	3	97/19	123/20			
	4	31/4	21/3			

Appendix I continued .....

Acc. No.	Pot No.	Plant number 1	2	3	4	5
124	1	96/18	18/3			
	2	15/7	42/11	8/3		
	3	78/10	47/10			
	4	14/6	13/4	50/33	83/30	
125	1	26/2	40/1	134/13	79/16	27/2
	2	29/8	90/13	34/9	77/16	
	3	63/13	73/12	5/1	171/23	
	4	12/5	13/3	109/26	18/2	173/22
126	1	24/14	14/0	13/4		
	2	17/10	25/10	2/1		
	3	21/10	70/38	51/18		
	4	54/4	7/0			
127	1	77/11	162/26			
	2	178/34	285/46			
	3	69/19	96/14			
	4	6/1	3/0	6/0		
128	1	37/11				
	2	2/1	33/9			
	3	72/31	9/4			
	4	9/3	11/4			
129	1	19/7	31/11			
	2	59/17	1/0			
	3	40/21	56/23			
	4	121/40	69/36			
130	1	5/0	2/0	2/1		
	2	66/30	21/6			
	3	4/0	6/2			
	4	14/8				
131	1	26/16	40/16	57/14		
	2	38/7	50/14	31/4		
	3	51/12	26/4	23/7		
	4	12/1	5/2	22/7		
132	1	140/11	210/11			
	2	37/6	48/5			
	3	33/11	119/40			
	4	202/11	146/5	54/2		
133	1	45/11	33/2			
	2	29/4				
	3	61/14	83/17			
	4	49/7	20/6			

Appendix I continued .....

Acc. No.	Pot No.	Plant number 1	2	3	4	5
134	1	39/8	13/0	19/4		
	2	35/8	41/6	27/6	61/14	
	3	94/18				
	4	29/6	16/0	24/3		
135	1	64/7	69/4			
	2	11/3	4/0			
	3	64/22				
	4	8/0	7/0	20/3		
136	1	68/14	32/4			
	2	43/7	82/19	46/11		
	3	77/14	33/10	73/19		
	4	60/16	7/1			
137	1	16/0	11/0	82/31		
	2	20/9	90/41	15/3	6/1	
	3	34/19	13/4	74/13		
	4	2/0	25/11			
138	1	1/1	2/1			
	2	11/1	2/0	57/6		
	3	7/2	3/0			
	4	57/19	40/8			
139	1	15/3	7/2	93/26	81/11	
	2	67/12	113/18	164/31	80/19	
	3	37/6	40/13			
	4	19/2	9/6	17/9		
145	1	5/0	27/3	3/0		
	2	7/2	19/6			
	3	7/1	2/1			
	4	10/1	18/5	2/0		
1830	1	33/6	21/11	5/2		
	2	44/8	11/1			
	3	80/21	17/3			
	4	22/4	20/4			
1832	1	34/11	34/4	4/1	6/2	
	2	11/1	16/1			
	3	37/8	5/0	11/1	46/18	
	4	11/4	19/4	13/0		
1845	1	47/9	3/0			
	2	43/10	32/6	31/9	46/6	21/4
	3	81/31	53/16			
	4	38/10	38/11	14/4		

Appendix I continued .....

Acc. No.	Pot No.	Plant number 1	2	3	4	5
213	1	35/0	30/0			
	2	1/0				
	3	63/0	37/0			
	4	31/0				
214	1	4/0	142/0	15/0		
	2	51/0	10/0			
	3	13/0	21/0	3/0		
	4	37/0	51/0			
215	1	591/0	178/0			
	2	80/0	13/0			
	3	9/0	11/0			
	4	94/0	28/0	25/0		
216	1	36/0	2/0	640/0	49/0	
	2	39/0				
	3	45/0	71/0			
	4	107/0	31/0	202/0		
217	1	183/0	6/0	156/0		
	2	207/0	107/0			
	3	53/0	37/0	4/0		
	4	594/0	18/0			
220	1	276/0	30/0	148/0		
	2	11/0	20/0			
	3	233/0	28/0			
	4	38/0	16/0	65/0		
221	1	20/0	11/0	26/0		
	2	8/0	13/0			
	3	120/0	38/0			
	4	91/0	50/0	72/0		
222	1	61/0	44/0			
	2	12/0	32/0			
	3	28/0	43/0			
	4	11/0				
223	1	136/0	78/0	34/0	4/0	
	2	66/0	47/0	15/0		
	3	16/0	1/0			
	4	19/0				
230	1	10/1	2/0	1/0		
	2	19/4				
	3	10/0	78/15	27/0	13/2	
	4	2/0	2/0	3/0	3/0	

Appendix I continued .....

Acc. No.	Pot No.	Plant number 1	2	3	4	5
231	1	41/5	159/21	96/14	86/7	
	2	17/4	187/66	16/0	83/19	
	3	114/38	104/25	24/7	47/7	151/43
	4	14/3	71/18			
232	1	56/14	169/36	292/109		
	2	48/16	41/19	38/11		
	3	14/3	116/41	207/61	19/3	
	4	8/2	138/7	15/6	5/1	
233	1	258/31	6/1			
	2	23/4	22/11	40/19		
	3	226/53	363/119			
	4	213/50	184/35			
234	1	2/0	1/0			
	2	327/26	232/19			
	3	58/14	1/0			
	4	4/1	53/8			
235	1	81/4	51/11			
	2	20/6	42/16	43/8		
	3	107/15				
	4	40/3	38/2	26/2	13/0	
236	1	14/1	15/1			
	2	53/1	53/3	58/4		
	3	37/2	100/6			
	4	52/4	80/10			
243	1	1/0	16/5	3/0		
	2	14/9	2/1			
	3	92/14	133/20			
	4	96/13	102/11	59/6	4/1	
1846	1	15/1	3/0	61/3		
	2	44/4	68/5	57/2		
	3	18/0				
	4	20/3	51/3			
255	1	19/8				
	2	6/1				
	3	11/5	8/3			
	4	37/11	47/21			
465	1	25/4	55/4			
	2	17/4	4/0	34/18	26/7	
	3	13/2	17/2			
	4	28/16	3/1	9/4	10/4	

Appendix I continued .....

Acc. No.	Pot No.	1	2	3	4	5
1682	1	58/11	87/13	12/6		
	2	8/1	18/5			
	3	52/13	12/3			
	4	6/1	20/4	1/0	11/1	21/3
493	1	106/7	74/4			
	2	9/1	11/1	26/4		
	3	-	-	-	-	-
	4	-	-	-	-	-
497	1	37/6	24/4			
	2	15/3				
	3	68/10	5/1	24/3		
	4	71/19	36/6	32/3		
504	1	6/1	3/1	23/6		
	2	64/13	31/4			
	3	19/1	33/3	17/1		
	4	13/2				
505	1	587/92	55/12			
	2	84/14	43/6	6/1		
	3	67/4	37/2	37/7		
	4	10/0	84/6	34/12		
506	1	2/0				
	2	4/2	16/4	26/11		
	3	64/18				
	4	64/13	12/5	15/7	72/22	
507	1	42/11	46/12	71/9		
	2	9/1				
	3	67/4	37/2	37/7		
	4	44/26	28/11			
508	1	47/3	94/19	20/1		
	2	25/4	37/2			
	3	16/4	26/11			
	4	44/9	52/13			
509	1	28/0				
	2	57/0	5/0			
	3	47/0	112/0			
	4	6/0				
1838	1	96/16	72/14			
	2	333/29	125/9			
	3	80/31	62/29	66/24	23/11	
	4	113/31	225/62	11/4		

Appendix I continued .....

Acc. No.	Pot No.	1	2	3	4	5
1839	1	24/0	17/8	108/33		
	2	137/18	29/6	126/22		
	3	6/0	1/0			
	4	197/53	46/11	16/7	164/41	
1840	1	41/4	83/14			
	2	10/3	9/2			
	3	137/16				
	4	82/18	112/16	37/4		
1841	1	69/11	42/4			
	2	89/8	34/13			
	3	2/0	4/0			
	4	6/0	46/11	63/4		
554	1	36/0	8/0	87/0		
	2	19/3	33/5			
	3	36/0	13/0			
	4	73/7				
556	1	22/0	85/0			
	2	43/5				
	3	8/0				
	4	21/5				
585	1	4/0	2/0			
	2	12/0	94/0			
	3	5/0	14/0	13/0		
	4	3/0	5/0			
586	1	51/0	36/0			
	2	28/0	26/0			
	3	32/4	5/1			
	4	6/1	3/0	3/1		
1529	1	129/18	203/32	43/6	60/9	15/2
	2	3/1				
	3	16/8				
	4	3/0				
1544	1	28/2	21/1	63/1	94/6	
	2	4/0	6/0			
	3	3/0	5/1			
	4	9/2	11/2	2/0		
1549	1	31/0				
	2	52/8	33/3			
	3	60/11	11/3			
	4	43/4	8/2	51/5		

Appendix I continued .....

Acc. No.	Pot No.	1	2	3	4	5
1641	1	16/0	19/0			
	2	27/0	53/0			
	3	7/0				
	4	25/0	34/0			
1712 (Flat)		23/6	28/6	16/1		
1563	1	104/13				
	2	243/29				
1684 (Flat)		51/6				
Can X Hex Sat						
	1	14/2	22/8			
	2	18/4	13/5			
	3	23/4				
	4	8/0				
Hex Sativa						
	1	219/23	41/11	29/5		
	2	39/8	15/5	63/12		
	3	63/9	19/8	43/9	88/17	
	4	50/10	29/8	70/11		
223a	1	87/6	34/2			
	2	355/14				
	3	38/4	156/8	54/4		
	4	84/7	95/6	86/6	61/5	
20 DRC		24 plants tested Average = 32.5% infested seed				

Appendix 2. Field data on infestation of Medicago carstiensis.

Vial No.	Strain No.	No. pods	No. insects male	No. insects female	No. seeds	Infested seeds	% Infestation
1	44	3	2	2	31	2	6.5
2	44	4	2	2	39	1	2.6
3	44	4	2	1	44	4	9.1
4	44	3	2	2	37	0	0.0
5	45	4	2	2	41	3	7.3
6	45	3	2	2	29	4	13.8
7	45	4	2	2	39	2	5.1
8	45	3	2	1	33	2	6.1
9	20 DRC	2	2	1	11	4*	36.4
10	S.W.	3	2	1	6	4	66.7

* All from one pod.

Appendix 3. Field data on infestation of Medicago dzhawakhetica.

South cage	Seeds	Infested seeds	% Infestation
Plant 1	161	13	8.1
Plant 2	182	9	4.9
Plant 3	121	11	11.0
Control (20 DRC)	204	58	28.4
		Strain mean = 8.0	
North cage			
Plant 1	123	15	12.1
Plant 2	197	23	11.7
Control (20 DRC)	187	53	28.3
		Strain mean = 11.9	

Appendix 4a. Data from perennial cross 217 X Sask.

F_2 Plant No.	Pod shape	No. curls	Total seeds	Infested seeds
1	T	1.0 - 1.5	19	1
2	T	1.5 - 2.0	60	4
3	T	1.0 - 1.5	4	0
4	T	2.5 - 3.0	66	4
5	T	2.0 - 2.5	94	5
6	T	2.0 - 2.5	100	8
7	T	1.5 - 2.0	57	4
8	T	1.0 - 1.5	18	3
9	T	1.5 - 2.0	55	3
10	T	1.5 - 2.0	19	2
11	T	2.0 - 2.5	100	11
12	T	1.5 - 2.0	44	6
13	T	2.0 - 2.5	100	4
14	T	1.5 - 2.0	70	8
15	T	1.0 - 1.5	100	13
16	T	1.0 - 1.5	24	3
17	T	1.5 - 2.0	83	9
18	T	1.0 - 1.5	100	16
19	T	1.0 - 1.5	61	5
20	T	2.0 - 2.5	40	2
21	T	1.0 - 1.5	34	6
22	T	2.0 - 2.5	43	1
23	T	1.5 - 2.0	100	11
24	T	2.5 - 3.0	25	0
25	T	1.5 - 2.0	57	8
26	T	2.5 - 3.0	70	3
27	T	2.5 - 3.0	78	8
28	T	1.5 - 2.0	32	5
29	T	1.0 - 1.5	28	5
30	T	1.0 - 1.5	49	5
31	T	2.0 - 2.5	19	1
32	T	1.0 - 1.5	93	13
33	T	2.5 - 3.0	60	1
34	T	2.0 - 2.5	38	2
35	T	1.5 - 2.0	31	0
36	T	1.0 - 1.5	43	9
37	T	2.5 - 3.0	21	1
38	T	1.0 - 1.5	100	18
39	T	1.0 - 1.5	88	14
40	T	2.0 - 2.5	30	5

Appendix 4a continued .....

F_2 Plant No.	Pod shape	No. curls	Total seeds	Infested seeds
41	T	2.0 - 2.5	52	4
42	T	2.5 - 3.0	47	2
43	T	1.0 - 1.5	23	4
44	T	1.5 - 2.0	58	6
45	T	2.5 - 3.0	100	6
46	T	1.0 - 1.5	16	5
47	T	1.0 - 1.5	37	8
48	T	2.0 - 2.5	50	2
49	T	1.5 - 2.0	38	5
50	T	1.0 - 1.5	51	7
51	T	1.5 - 2.0	100	11
52	T	1.0 - 1.5	59	7
53	T	2.0 - 2.5	67	2
54	T	1.0 - 1.5	46	8
55	T	1.0 - 1.5	40	11
56	T	2.5 - 3.0	8	0
57	T	1.5 - 2.0	81	13
58	T	1.0 - 1.5	27	11
59	T	1.0 - 1.5	31	8
60	T	1.5 - 2.0	49	3
61	T	2.0 - 2.5	100	7

Appendix 4b. Data from perennial cross 217 X 1682.

F_2 Plant No.	Pod shape	No. curls	Total seeds	Infested seeds
1	T	2.5 - 3.0	43	5
2	T	2.0 - 2.5	8	0
3	T	2.5 - 3.0	31	2
4	T	2.0 - 2.5	19	2
5	O	2.0 - 2.5	8	2
6	T	1.5 - 2.0	19	4
7	T	2.0 - 2.5	17	1
8	T	1.5 - 2.0	25	4
9	T	1.5 - 2.0	18	1
10	T	2.0 - 2.5	13	2
11	O	2.5 - 3.0	38	3
12	T	1.5 - 2.0	3	0
13	O	1.0 - 1.5	10	4
14	T	1.5 - 2.0	14	2
15	T	1.5 - 2.0	16	2
16	T	2.5 - 3.0	49	4
17	T	2.5 - 3.0	32	1
18	O	1.0 - 1.5	22	6
19	T	2.0 - 2.5	12	1
20	T	1.5 - 2.0	23	4
21	O	2.0 - 2.5	28	5
22	T	1.5 - 2.0	17	2
23	T	2.0 - 2.5	19	2
24	T	1.5 - 2.0	21	4
25	O	1.5 - 2.0	34	9
26	O	1.0 - 1.5	40	7
27	T	2.0 - 2.5	37	4
28	T	1.0 - 1.5	27	5
29	O	1.0 - 1.5	22	5
30	T	1.5 - 2.0	29	3
31	T	2.0 - 2.5	19	1
32	T	1.0 - 2.0	24	3
33	T	2.0 - 2.5	37	3
34	T	2.0 - 2.5	25	4
35	O	1.5 - 2.0	40	6
36	O	1.0 - 1.5	12	2
37	O	1.5 - 2.0	15	5
38	T	2.0 - 2.5	5	0
39	T	1.5 - 2.0	14	3
40	O	1.5 - 2.0	35	8
41	T	2.0 - 2.5	30	2
42	O	1.0 - 1.5	22	5
43	T	1.5 - 2.0	34	7
44	T	1.5 - 2.0	17	2

Appendix 4c. Data from perennial cross 217 X 125.

F_2 Plant No.	Pod shape	No. curls	Total seeds	Infested seeds
1	T	1.0 - 1.5	45	7
2	O	0.5 - 1.0	23	13
3	O	0.0 - 0.5	48	13
4	T	1.0 - 1.5	31	5
5	T	1.0 - 1.5	26	8
6	O	0.5 - 1.0	19	7
7	T	1.0 - 1.5	58	13
8	O	0.0 - 0.5	34	11
9	T	1.0 - 1.5	64	18
10	O	0.5 - 1.0	9	1
11	T	1.5 - 2.0	12	2
12	T	1.0 - 1.5	30	7
13	O	0.0 - 0.5	53	10
14	O	0.0 - 0.5	22	4
15	O	0.5 - 1.0	20	5
16	T	1.5 - 2.0	66	6
17	T	1.0 - 1.5	36	3
18	O	0.5 - 1.0	40	11
19	O	0.0 - 0.5	33	12
20	T	1.0 - 1.5	29	5
21	O	0.5 - 1.0	32	8
22	T	1.0 - 1.5	64	8
23	T	1.0 - 1.5	32	6
24	O	0.5 - 1.0	25	4
25	O	0.5 - 1.0	25	7
26	O	0.5 - 1.0	49	7
27	T	1.0 - 1.5	55	13
28	O	0.5 - 1.0	29	9
29	T	1.5 - 2.0	14	3
30	T	1.5 - 2.0	34	6
31	T	1.0 - 1.5	75	17
32	O	0.5 - 1.0	53	14
33	T	1.0 - 1.5	36	8
34	O	0.5 - 1.0	43	7
35	O	0.5 - 1.0	52	9
36	T	1.0 - 1.5	36	3
37	T	1.5 - 2.0	100	22
38	O	0.0 - 0.5	57	14
39	T	1.0 - 1.5	42	12
40	O	0.5 - 1.0	28	4

Appendix 4c continued .....

F_2 Plant No.	Pod shape	No. curls	Total seeds	Infested seeds
41	O	0.5 - 1.0	35	8
42	O	0.5 - 1.0	30	5
43	T	1.0 - 1.5	21	4
44	O	0.5 - 1.0	7	0
45	T	1.5 - 2.0	48	2
46	O	0.5 - 1.0	25	6
47	O	0.5 - 1.0	38	11
48	T	1.5 - 2.0	40	9

Appendix 4d. Data from perennial cross 217 X 1529.

F_2 Plant No.	Pod shape	No. curls	Total seeds	Infested seeds
1	O	0.5 - 1.0	78	8
2	O	1.0 - 1.5	54	4
3	T	1.0 - 1.5	14	3
4	O	0.5 - 1.0	100	14
5	O	0.5 - 1.0	16	6
6	O	1.5 - 2.0	53	8
7	T	1.0 - 1.5	38	4
8	O	1.0 - 1.5	34	6
9	O	1.5 - 2.0	23	2
10	T	1.0 - 1.5	18	4
11	T	2.0 - 2.5	38	2
12	T	2.0 - 2.5	32	2
13	T	1.5 - 2.0	58	7
14	O	1.5 - 2.0	55	13
15	T	1.0 - 1.5	61	8
16	O	2.0 - 2.5	31	8
17	T	1.5 - 2.0	14	0
18	O	1.0 - 1.5	39	7
19	T	1.5 - 2.0	57	11
20	T	2.5 - 3.0	100	4
21	T	1.0 - 1.5	45	6
22	T	2.0 - 2.5	47	3
23	T	1.5 - 2.0	58	5
24	O	1.0 - 1.5	33	9
25	O	1.0 - 1.5	27	11
26	T	1.5 - 2.0	33	6
27	O	1.5 - 2.0	52	10
28	T	2.0 - 2;5	21	1
29	O	1.0 - 1.5	45	5
30	O	1.5 - 2.0	29	7
31	T	2.5 - 3.0	100	9
32	T	1.5 - 2.0	58	6
33	T	2.0 - 2.5	74	10
34	O	1.5 - 2.0	16	5
35	O	1.5 - 2.0	28	5
36	T	2.0 - 2.5	54	4
37	O	1.0 - 1.5	48	12
38	T	1.5 - 2.0	100	14
39	T	2.0 - 2.5	30	2
40	O	1.0 - 1.5	42	4
41	T	1.5 - 2.0	23	4
42	T	2.0 - 2.5	68	5
43	O	1.0 - 1.5	25	6
44	O	1.0 - 1.5	11	1
45	T	1.0 - 1.5	45	2
46	T	2.0 - 2.5	19	0
47.	O	1.0 - 1.5	40	9

Appendix 5. Data from infestation of Medicago coerulea and other perennials in individual raceme cages.

Vial No.	Accession No.	Strains		Control (20 DRC)	
		Total seeds	Infested seeds	Total seeds	Infested seeds
1	213	19	0	41	5
2	213	26	3	-	-
3	214	19	1	38	9
4	214	17	4	-	-
5	217	12	0	43	6
6	217	22	3	-	-
7	216	25	2	30	11
8	216	9	1	-	-
9	223a	33	7	28	8
10	223a	29	7	-	-
11	215	27	0	19	6
12	215	20	2	-	-
13	507	14	1	55	14
14	507	11	4	-	-
15	221	41	3	38	13
16	221	43	4	-	-
17	220	19	0	50	6
18	220	26	2	-	-
19	223a	20	4	31	8
20	223a	13	9	-	-
21	222	22	2	23	9
22	222	20	4	-	-
23	220	19	0	26	4
24	220	14	1	-	-
25	214	29	2	33	7
26	214	24	3	-	-
27	222	18	0	39	11
28	222	23	0	-	-
29	215	9	0	27	13
30	215	15	2	-	-
31	215	16	-	22	10
32	215	24	3	-	-
33	223	32	0	29	6
34	223	21	6	-	-
35	223	22	0	20	0
36	223	20	0	-	-
37	507	19	4	37	3
38	507	19	8	-	-
39	507	37	0	39	16
40	507	30	4	-	-
41	125	22	8	26	11
42	125	23	13	-	-
43	125	17	4	26	4
44	125	16	7	-	-
45	216	26	0	38	11

Appendix 5 continued.....

Vial No.	Accession No.	Strains		Control (20 DRC)	
		Total seeds	Infested seeds	Total seeds	Infested seeds
46	216	20	2	-	-
47	220	26	2	19	10
48	220	28	0	-	-
49	217	34	0	23	12
50	217	28	3	-	-
51	217	31	0	33	11
52	217	30	2	-	-
53	509	32	2	41	17
54	509	22	8	-	-
55	216	16	3	39	12
56	216	20	3	-	-
57	213	11	0	53	14
58	213	18	4	-	-
59	223	31	1	47	11
60	223	28	7	-	-
61	221	15	0	39	0
62	221	23	0	-	-
63	214	20	0	27	4
64	214	17	5	-	-
65	125	20	8	47	8
66	125	17	14	-	-
67	221	16	0	44	16
68	221	11	4	-	-
69	213	21	0	33	16
70	213	15	2	-	-
71	222	16	3	38	15
72	222	20	5	-	-
73	223a	33	7	40	9
74	223a	30	9	-	-
75	216	17	0	39	16
76	216	19	2	-	-
77	214	29	0	51	16
78	214	25	3	-	-
79	220	21	1	32	14
80	220	26	3	-	-
81	509	19	0	37	9
82	509	23	2	-	-
83	125	22	6	37	5
84	125	32	17	-	-
85	223	28	4	29	7
86	223	28	8	-	-
87	222	18	0	27	3
88	222	23	4	-	-
89	222	26	0	41	0
90	222	29	3	-	-

Appendix 5 continued .....

Vial No.	Accession No.	Strains		Control (20 DRC)	
		Total seeds	Infested seeds	Total seeds	Infested seeds
91	125	21	9	21	11
92	125	18	8	-	-
93	221	31	4	37	14
94	221	18	5	-	-
95	509	16	3	23	17
96	509	19	11	-	-
97	215	22	1	29	9
98	215	2o	10	-	-
99	509	23	1	33	19
100	509	22	8	-	-
101	507	13	0	26	14
102	507	21	16	-	-
103	223a	14	7	31	11
104	223a	17	12	-	-
105	217	22	1	30	14
106	217	22	5	-	-
107	215	20	3	25	8
108	215	18	6	-	-
109	213	26	0	28	6
110	213	20	6	-	-
111	507	25	4	44	17
112	507	28	5	-	-
113	223a	22	13	33	10
114	223a	24	11	-	-
115	509	24	2	38	13
116	509	27	5	-	-
117	217	31	2	33	23
118	217	30	2	-	-
119	214	8	0	30	17
120	214	14	3	-	-
121	216	28	4	27	21
122	216	24	11	-	-
123	221	19	2	37	23
124	221	25	6	-	-
125	220	32	4	32	20
126	220	26	5	-	-
127	223	30	5	24	18
128	223	30	9	-	-
129	213	24	11	34	21
130	213	30	12	-	-
131	220	22	3	25	16
132	220	24	7	-	-
133	221	19	5	22	12
134	221	23	7	-	-
135	222	11	2	34	23
136	222	16	5	-	-
137	223	19	9	27	11
138	223	17	7	-	-

Appendix 6. Original data of infestation in annuals.

Accession No.*	I	II	III	IV	Total	Mean % infestation
19	4/11	4/14	3/17	1/11	12/53	22.6
37	0/21	0/22	0/12	0/16	0/71	0.0
42	0/8	0/10	0/6	0/6	0/30	0.0
54	0/11	0/9	0/10	0/8	0/38	0.0
61	2/14	3/18	1/11	2/11	8/54	14.8
70	0/12	0/9	0/11	0/14	0/35	0.0
237	0/19	0/9	0/9	0/4	0/4o	0.0
248	0/8	0/13	0/8	0/10	0/39	0.0
254	0/9	0/13	0/13	0/10	0/45	0.0
261	0/7	0/13	0/9	0/5	0/34	0.0
294	1/23	3/19	1/19	1/18	6/79	7.8
324	5/19	1/10	0/8	2/10	8/47	17.0
253	0/4	0/11	0/9	0/8	0/32	0.0
270	2/14	1/11	0/7	2/14	5/46	10.9
418	5/13	3/7	3/10	4/9	15/39	38.4
480	0/12	0/17	0/15	0/10	0/54	0.0
539	0/13	0/16	0/11	0/11	0/51	0.0
542	0/5	0/4	0/7	0/9	0/25	0.0
553	1/8	2/11	1/13	0/6	4/38	10.5
563	5/9	3/7	1/7	2/7	11/30	36.7
567	0/5	0/11	0/9	0/8	0/33	0.0
571	0/11	0/10	0/5	0/8	0/34	0.0
675	0/2	0/7	0/7	0/10	0/26	0.0
676	0/7	0/4	0/8	0/4	0/23	0.0
684	2/13	3/15	4/10	0/7	9/45	20.0
713	0/3	0/11	0/7	0/10	0/31	0.0
769	0/11	0/8	0/10	0/4	0/33	0.0
795	2/10	1/12	1/8	2/13	6/43	14.0
855	0/8	0/12	0/10	0/9	0/39	0.0
865	0/11	0/7	0/7	0/9	0/34	0.0
869	0/4	0/7	0/5	0/9	0/25	0.0
1306	2/9	1/14	4/12	2/10	9/45	20.0
1338	0/8	0/9	0.9	0/11	0/37	0.0
1499	1/6	2/13	2/11	3/11	8/41	19.5
1609	0/8	0/13	0/8	0/9	0/38	0.0
Control (20 DRC) Average of 3 plants						25.5%

* Accession number refers to Medicago collection at the Department of Genetics, University of Alberta, Edmonton, Alberta

** Numerator = infested seeds; denominator = total seeds observed.